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BORDER PINING IN SHEEP.—I.

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INTRODUCTION.

"NORTHUMBRIAN PINING" may be defined as a chronic, wasting disease of hill sheep, characterised by anæmia and emaciation. It is a disease of some antiquity and probably received its name from the Anglo-Saxon word pinian, to torment, or waste away under pain. Hogg[1] has recorded acute outbreaks of pining, but as observed to-day the onset of the disease is slow, and it runs a protracted course resulting in months of unthriftiness and frequently ending in death from exhaustion or broncho-pneumonia. Animals which recover are very liable to a second attack the following year. No satisfactory pathological account of pining has hitherto appeared in the literature.

Synonyms.—Pine, Vinquish, Daising.

History.

The earliest written account of pining is from the pen of James Hogg, the Ettrick Shepherd (1770-1835), who contributed his classical account of the disease to the *Quarterly Journal of Agriculture* in 1831.[1] It is evident that Hogg regarded pining as a specific malady of hill sheep, characterised by lassitude, unthriftiness, constipation and anæmia, and he stated that it was a new disease on the borders, although he was aware of its existence in Galloway and also in some districts of the middle Highlands. Both Hogg and the Editor of the above Quarterly drew attention to its widely spread and serious nature, recording that the disease might suddenly attack a "whole flock," and "if the shepherd has not the means of changing the pasture, all those affected will fall in the course of a month." Hogg did not regard pining as a contagious disease, but on the contrary, considered that it proceeded wholly from the food, and added that the general belief among stepherds was that the extirpation of the common

mole (*Talpa europœa*) from hill pastures, presumably by altering the nature or quality of parts of the grazings, was the primary cause of the stock pining.

Hogg further stated that there was only one effective cure for pining, which was to change the pasture to one of more succulent herbage. " If ever the bowels are open and marks of a slight flux appear then the animal is safe and the necessary change has taken place in the constitution. Nevertheless these sheep will be the first to take it again. They are sure to take it again either that year or the next; so that upon the whole a farm cannot be subject to a more ruinous distemper. Few of the ewes that are affected in autumn have lambs next year or they have them far too late to be of any value."

More recently McGowan and Smith[2] recorded their observations on pining as seen on farms situated on the Scottish side of the Cheviot Hills. These authors noted the extreme anæmia exhibited by pining sheep, but failed to detect any characteristic abnormalities on *post-mortem* examination. They definitely excluded intestinal parasites and liver flukes from their consideration of the cause " because none may be present in typical cases." During their enquiries emphasis was always laid on the occurrence of pining on farms situated on the porphyritic rock lying on the Scottish side of the Cheviots, and extending southwards, especially into the Coquet Valley. Reference was also made to the general opinion that the change of grazing, already mentioned, should be made to pasturage situated above sandstone or limestone. It was concluded by these authors, however, that pining was not limited to any special type of rock or soil, and evidence was obtained that even changing the sheep from one pining farm to another gave good results. With regard to the cause of pining McGowan and Smith arrived at a conclusion not unlike that of Hogg, namely, that pining was the result of a nutritional defect, and stated that " the sheep on pining farms limit themselves to certain parts of the pasturage, and will not feed on the rest unless compelled to. The result is their range of feeding is limited, hence they do not obtain sufficient variation, their diet is insufficient and monotonous, and pining follows." It was considered that the beneficial results of changing the sheep were due to better and wider ranging over the new ground. These authors suggested that deficiency of an accessory food factor might operate in causing pining, although it was realised that certain facts did not accord with this view, such as the prevalence of pining in wet seasons, and its occurrence during the summer months when the nutritional value of herbage is best.

As regards the gravity of the disease McGowan and Smith refer to the common belief among farmers, that on these pining farms the whole stock would die out in less than two years if the sheep were kept constantly on the ground.

Occurrence.

In Northumberland the disease is known to occur in Bowmont Water, College Valley, and Coquetdale, all of which are valleys situated among the Cheviot foothills. The other parts of the county are regarded as being free from specific pining. According to the Editor of the *Quarterly Journal of Agriculture* (*loc. cit.*) pining prevailed on the green pastures of the Cheviots, on the hills running through the south-western parts of Roxburghshire, the pastoral districts of Selkirk and Peeblesshire, and in some other districts in Scotland, including Galloway. McGowan's investigation was carried out in 1921, and it was found that the disease was best known in the Cheviot area.

Later Views on Pining, and its Co-relation with Deficiency Diseases Occurring in Different Parts of the World.

In 1928 Godden and Grimmett[3] drew attention to the similarity of border pining to a disease of sheep and cattle in New Zealand, termed " bush sickness." Reference was made to Aston's work,[4] which showed (*a*) that bush sickness resulted from stock grazing on pastures with an abnormally low content of iron, although satisfactory in other respects, and (*b*) that the administration of iron ammonium citrate cured the extreme anæmia characteristic of bush sickness.

Following on McGowan's observations an investigation was undertaken at the Rowett Institute into the relative mineral content of pasture from pining and non-pining areas, and in general the result showed the iron content was lower in the pining than in the non-pining herbage.[3] Later, Orr[5] recorded that pining may occur on soils rich in iron, but suggested some factor which interfered with the absorption of iron by the plant.

The curative effects of iron salts have also been observed by Orr[6] in the treatment of a disease of working oxen prevalent in the Nakuru district of Kenya Colony. In its clinical manifestations this disease—so-called Nakuruitis—closely resembles bush sickness, and in preliminary tests Orr found that animals allowed to lick a mixture of equal parts of common salt and ferric oxide remained healthy, while control animals, on the same pasture, but without the salt lick, developed typical symptoms of the disease. Orr[7] concluded that border pining was very similar to, if not identical with, the bush sickness of New Zealand and " Nakuruitis " of Kenya, and stated that the administration of soluble iron salts to pining sheep seemed to act as a cure. In two reviews of iron-deficiency diseases published in 1931 and 1932, Aston[8, 9] reaffirmed the above analogy, and carried it a step further by grouping these three diseases together with the so-called " salt sickness " of Florida[10]—described as a nutritional anæmia, which responds to cattle licks composed of ferric oxide, copper

sulphate and common salt—as specifically due to deficiency of iron in the pastures.

The publication of these views and in general the increase in knowledge concerning the rôle of minerals in animal nutrition encouraged the belief that Northumbrian pining might be prevented by allowing the sheep access to mineral supplements in the form of licks. The provision of licks to supply a deficiency in the food of animals must be regarded as a somewhat inexact method, since it cannot be regulated to ensure that the most needful individuals will consume adequate amounts. Hill sheep, however, are seldom or never trough-fed, and the practice of placing iron-containing licks on sheep runs has commended itself to border flockmasters on the grounds of simplicity. After a trial of these licks extending to eight or ten years, the consensus of opinion, so far as it has been ascertained by the writers, seems to be that these licks have failed to influence the incidence and course of the naturally occurring disease, and, in the opinion of farmers, no treatment has yet been discovered which obviates the necessity for periodic changing of the stock.

In 1933 Greig and Dryerre,[11] in collaboration with Godden, Crichton and Ogg, recorded the results of an investigation into a disease called " pine " occurring among sheep and young cattle grazed continuously on certain parts of the Island of Tiree. The symptoms are stated to be progressive debility, anæmia and emaciation, and, as in border pining, the trouble may be entirely prevented by a timeous change to pastures situated on the raised beach terrain. The administration of crude ferric oxide appeared to be of specific curative and preventive value, and accordingly the authors concluded that it was probable that the cause of pine is a deficiency of iron in the pasture and/or of " available " iron in the soil of the affected areas. The iron-content of the Tiree soils was examined by the method employed by Askew and Rigg[12] in New Zealand, *i.e.*, extraction with 5 per cent. oxalic acid, when it was found that whereas iron was easily extracted from the soil from the " non-pine " areas, repeated extractions were necessary before iron could be determined in the soil from the " pine " areas. Greig and Dryerre[11] *et al.* discussed the probable significance of this finding, but were unable to arrive at any definite conclusion. *Post-mortem* findings on affected cattle were not recorded.

Clinically, Tiree pine appears to resemble closely border pining, bush sickness, etc., yet in the writers' opinion it is doubtful whether or not, in the absence of precise pathological data and *post-mortem* records, the symptoms above recorded should have been regarded as diagnostic of a deficiency disease, because similar symptoms are also exhibited in other common bovine and ovine diseases, such as Johne's disease and gastro-intestinal parasitism.

Diseases in the North of England which Simulate Pining.

In Northumberland the term " pining " is current in the sheep breeding and feeding areas, but its use is attended by a curious distinction. Thus, in uplands such as Coquetdale, College Valley and Bowmont Water, pining denotes the malady first described by Hogg, and among the farming community is still regarded as a distinct disease of obscure causation, characteristic of farms in these areas. On lower-lying sheep farms, on the other hand, a proportion of lambs commonly become unthrifty during the summer and autumn months. This unthriftiness is frequently termed " pining " or " pine," but in this case it is quite usual to suspect the ravages of gastro-intestinal parasites. (Everywhere, except in these upland areas, where the name carries its specific meaning, pining means unthriftiness from any cause, and, for example, is frequently applied to cattle suffering from Johne's disease or tuberculosis.) The symptoms in both types of pining are similar, if not identical, and the writers who have had the opportunity of examining sheep said to be typical cases of both diseases, have been struck by their apparent similarity. In view of the growing recognition of the importance of gastro-intestinal parasites in causing wastage among hill flocks, the question has arisen as to how far it is necessary or justifiable to separate two diseases which, clinically at least, bear such a close resemblance.

It is of interest to note that in two adjacent Northumbrian valleys, namely Tynedale and Redewater, which are not regarded as pining areas, a similar kind of disease is common. Locally, the condition is known as " blood rot," and it attacks Blackface and Cheviot ewes, being most prevalent during June and July, when 5-10 per cent. of the stock may be affected. Blood rot was formerly thought to be due to debility from heavy suckling of the lamb, but that this is not so is shown by the fact that one of us (W. L. S.) has observed the disease in hoggs and also in eild ewes. The symptoms in blood rot are severe anæmia and emaciation, with or without superpurgation prior to death, and it can be distinguished from border pining by its much more rapid course and termination. The senior author has examined 14 cases of blood rot and in each found heavy infestations with gastro-intestinal parasites, chiefly *Hæmonchus contortus*. As a result of this discovery the greatest benefit has followed the use of suitable anthelmintics, *e.g.*, copper sulphate and carbon tetrachloride. (Unpublished data.) It is of interest to note that the old cure was to change affected animals to more succulent herbage and especially to young growing corn.

Similarly, in parts of Cumberland the term " Saut " is applied to a local malady of hill sheep. *Post-mortem* examination of affected sheep from two different flocks has revealed that, in all probability, the cause of the disease is gastro-intestinal parasites, chiefly *Ostertagia circumcincta*. These enquiries into local diseases

of sheep have clearly demonstrated the fact, hitherto not clearly comprehended, that nematode parasites of one species or another occur commonly among hill sheep, and that they not infrequently cause disease.

In conclusion, judging from the literature and from preliminary enquiries among flockmasters, it seems that the influence of gastro-intestinal parasites in causing pining has been insufficiently studied. On the contrary, the tendency has been to link these troubles together as manifestations of nutritional deficiencies, on what, in the writers' view, is perhaps slender and incomplete evidence. The fact that a horse's condition may improve under a course of arsenic therapy does not prove that the animal was suffering from a deficiency of arsenic, and this argument holds with equal force in the case of iron. Previous investigations seem to have been undertaken mainly from the standpoint of the agricultural chemist, and *post-mortem* records of affected cases are meagre in the extreme. The writers therefore agree with Malcolm,[13] that in the absence of pathological data, blood and hæmoglobin determinations, etc., the etiology of several of these diseases cannot be held to have been proven. Under-nutrition or malnutrition are important predisposing causes of parasitic disease in livestock, but it must not be overlooked that certain alimentary parasites *per se* produce very definite diseases, and that sheep are particularly liable to such infestations. It therefore seems unjustifiable to ignore their possible influence in causing anæmia and emaciation in view of their acknowledged importance in sheep husbandry, while to state that " none may be present " in emaciated sheep in Britain suggests that sufficient care has not been paid to their detection.

The present investigation was undertaken at the request of border flockmasters, and several offered certain facilities for experiment. In what follows the experimental work will be outlined, and the lesions in 13 typical cases of pining will be recorded and briefly discussed.

Experimental.

The system of changing, whereby the gross effects of Northumbrian pining are ordinarily combated, masks the incidence and course of the natural disease, and for the purposes of this enquiry it was decided to set up experimental units of healthy and pining sheep. By keeping these animals under observation on affected farms, and by withholding the customary change from the pining group, it was anticipated that data on the naturally occurring disease would be obtained. Finally, as the disease progressed, it was designed to submit as many of these animals as possible to a *post-mortem* examination.

Blood and fæces samples were obtained regularly from these sheep and submitted to the following examinations :—

I. Enumeration of red blood cells.
II. Enumeration of white blood cells.
III. Estimation of percentage volume of red cells.
IV. Calculation of average individual cell volume.
V. Estimation of hæmoglobin.
VI. Establishment of colour index.
VII. Differential leucocyte counts.
VIII. Estimation of serum calcium.
IX. Estimation of blood inorganic phosphorus.
X. Fæcal egg counts.

Technique.—The blood and fæces samples were obtained in the field and transferred to the laboratory. Potassium oxalate was used as the anti-coagulent, but differential leucocyte counts were made on smears of fresh unoxalated blood. For the enumeration of the red and white blood cells, Potain pipettes were used, and the counting was done on Thoma hæmocytometers. For the red cell count the blood was diluted with 1 per cent. formalin in normal saline, and 4 per cent. acetic acid was used as the diluent for the white cell count.

The red cell volume, or the volume per cent. occupied by the red corpuscles, was measured by centrifuging oxalated blood in graduated tubes until constant readings were obtained. The average individual cell volume was calculated by Murphy and FitzHugh's method,[14] whereby the percentage volume of red cells is divided by the number of red cells in 100 c.c. blood, being expressed as cubic centimetres $\times 10^{-11}$, *i.e.*:—

$$\frac{\text{percentage vol. red cells}}{\text{number of R.B.Cs. in 100 c.c. blood}} = \text{Average individual cell volume (in c.cms.} \times 10^{-11}\text{).}$$

Discarding the negative power, the average figure for normal sheep in this laboratory has been found to be approximately 3·07. These workers found that this calculation was useful in differentiating between acute and chronic anæmias, and that the information obtained was as reliable as that afforded by measurement of the mean cell diameter. An increase of from 20 to 100 per cent. was recorded in most cases of pernicious anæmia in the human subject. Hæmoglobin was estimated against a standard Newcomer disc, using a Klett colorimeter. The results were expressed in grammes hæmoglobin per 100 cubic centimetres blood, and were afterwards transferred to a sheep scale constructed by Piercy,[15] who showed that the average amount of hæmoglobin in normal sheep blood was 11·45 grammes per cent. By expressing this normal quantity of hæmoglobin as the figure 100, deviations from normality are easily seen. The colour index of the blood was obtained by dividing the hæmoglobin expressed as grammes per 100 c.c. blood by the number of red blood cells in millions per cubic millimetre. Using Piercy's hæmoglobin figure and adopting Gaiger and Davies'[16] value of 10·3 millions

for the denominator, a normal colour index of 1·11 is obtained. Hæmatological work in this laboratory indicates, however, that the number of erythrocytes in normal sheep blood is greater than 10·3 millions per cmm., more probably lying between 11 and 12 millions. Consequently, if the normal colour index is taken as 1, no great error will arise. In the differential counts 400 leucocytes were counted and the percentage of neutrophiles, lymphocytes, large monocytes, eosinophiles and basophiles noted.

Serum calcium was estimated by Kramer and Tisdall's[17] method, and inorganic phosphorus by a micro-adaptation of Brigg's modification of the original Bell-Doisy[18] technique. These results were expressed in milligrammes per cent. of serum and whole blood respectively. Stoll's[19] method was employed (after the first six months) to estimate the numbers of ova of parasites in the fæces. By this method the ova are expressed in hundreds per gramme fæces, the number being referred to as the egg index. The method in use at the commencement of the work was less accurate and more cumbersome than Stoll's, and yielded lower comparative figures.

Farm A.—Situated on Cheviot range of hills in a geological formation of reddish porphyritic lavas of the old red sandstone age. This indicates relative richness in ferric iron, more readily soluble than various other iron compounds such as magnetite, and from a general geological point of view there is nothing to suggest that the subsoil might be relatively deficient in iron.* This formation determines the nature of the subsoil, which is thin. The sheep walks consist of both gentle and abrupt slopes running to an elevation of 1,800 feet, and separated by narrow water courses in the valleys. The best pastures are closely grazed and comprise a variety of grasses, such as sheep's fescue, sweet vernal, Yorkshire fog and bent; bird's foot trefoil, yarrow and plantain were also present. At the other end of the scale there are large tracts composed almost entirely of coarse inedible grasses, such as blue molinea, mat grass and bent. In summer these grasses are tall and waving, and show no evidence of being grazed, since generally speaking they are too coarse and wiry to be relished by sheep, which, however, feed on the young spring shoots. The pastures and herbage on the southern slopes are better and finer than on the other exposures. There is little or no heather on Farm A. Three ewes are carried to five acres of land, but hoggs are wintered from home, and ewes are not mated until they are two-and-a-half years old. Farm A. is a reputed pining farm, but is considered healthy in other respects and the land is free from ticks (*Ixodes ricinus*).

In June, 1933, five pining ewes were selected by the shepherd

*For this information we are indebted to Mr. Serge Tomkeieff, of the Geological Department, Armstrong College.

and ear-tagged with numbers 1 to 5. Five non-pining and apparently healthy ewes were selected for comparison and ear-tagged with numbers 6 to 10. Initial samples of blood and fæces were collected, and these groups remained under observation for 18 months.

Results.—The results of the first analyses are recorded in Table I (Appendix). Reference to this table shows a striking difference in the data obtained from these two groups of sheep. The piners all suffered from anæmia, hæmoglobin and red cells being about half their normal values. The colour indices were not altered materially, but the egg indices are higher in the pining group. In all cases the serum, after centrifugalising, was perfectly clear and showed no evidence of hæmolysis. The figures obtained from the non-pining ewes, although low as judged by abattoir standards, are in all probability within the normal range.

Differential leucocyte counts were undertaken on June 22nd, and the results are recorded in Table II (Appendix). Fraser[20] has studied the cellular content of normal sheep blood, and his figures are appended for comparison. It may be noted that in both groups on Farm A., the differential counts differ considerably from Fraser's normal standards in showing a decreased percentage of neutrophiles, and a slight increase in lymphocytes and eosinophiles. This change is quite marked in Group II, which, however, were not regarded as normal controls, but merely as representative of the non-pining and presumably healthy sheep on this farm. Disturbances in normal leucocyte proportions have already been noted by Fraser (*loc. cit.*) and by Stewart and Piercy[21] in parasiticised sheep. Thus, in well-marked intestinal infestations with nematode worms these authors have observed an increase in the proportion of lymphocytes, and a decreased proportion of neutrophiles, with some increase in eosinophiles. These changes are marked in the ewes in Groups I and II.

Table I also records figures for serum calcium and blood inorganic phosphorus. The average figure for the five diseased sheep is 8·36 mgs. calcium and 4·97 mgs. phosphorus, and for the non-pining group 9·20 mgs. calcium and 5·35 mgs. phosphorus. In all probability the differences in these sets of figures are insignificant, but interesting and perhaps significant is the low general level of calcium in both groups. The physiological limits of serum calcium in sheep have not yet been determined, but healthy sheep grazing on enclosed pastures generally show a figure of between 10 and 11 mgs. per cent., and an average of 8·36 mgs. would probably be regarded by most workers as definitely low. (Stewart and Shearer.[22]) This probably reflects the poorer state of general nutrition shown by nearly all hill sheep at certain times of the year.

Davidson,[23] Orr[5] and many others have shown that massive doses of iron ammonium citrate elicit a marked response in the

treatment of certain types of anæmia, and reference has already been made to Orr's experiments, which showed that soluble iron salts seem to act as a cure in pining. It was therefore of interest to observe the effect of administering large doses of this iron salt to a group of five pining ewes on Farm A. These ewes were ear-tagged 11-15 and comprise Group III, Table I. Reference to this table shows these ewes to be less severely affected, and two animals do not show anæmia, although suspected by the shepherd to be in the initial stages of the disease. Eight grammes of iron ammonium citrate were thrown on the back of the tongue of these ewes every second day for two months, commencing July 17th. Blood studies over this period (Table V, Appendix) showed improvement of condition in every case. This was pronounced in the case of Ewe No. 13, where the red cell volume increased from 18 to 32 per cent., and in Ewe No. 15, which by January showed an even greater increase. On the other hand, three of the ewes in Group I (Table III) receiving no special treatment and from which even the customary change of pasture was withheld, showed a precisely similar increase in red cells and hæmoglobin. Sheep No. 5 is particularly noteworthy. On July 6th her blood figures were: R.B.Cs. 2·57 mill., Hb. 26 per cent. normal, red cell volume 12 per cent. When killed for *post-mortem* examination in November these figures were all increased by over 100 per cent.

The non-pining ewes in Group II maintained a constant to a slightly increased level of condition until December (Table IV). Both Groups II and III were given their customary autumn change of pastures, so it is impossible to say how far the improvement in Group III resulted from the administration of iron. The opinion is held, however, that the general improved condition for the ewes in all groups as the year advanced was more in the nature of a seasonal variation, and would have occurred irrespective of the iron treatment. In this connection it is well known that, speaking generally, all hill sheep advance in condition as the year lengthens, the peak being reached in mid-autumn or early winter. The complete blood pictures for Groups I, II and III are give in Tables III, IV and V, and they show, in general, that this improvement was not maintained after December, when the blood figures commenced to recede, coincident with the onset of harder winter conditions and changes in the nutritive value of the herbage.

(Cruikshank[24] and others have shown that the mineral content of pastures undergoes a definite seasonal variation, which was most clearly shown in Cruikshank's samples by the CaO, which rises to a maximum in mid-summer and then steadily falls.)

Low egg indices in Group II ewes were recorded throughout the experiment, but in Groups I and III the egg index is seen to vary inversely with the hæmoglobin values. The writer agrees

with Taylor[25] that the egg index, particularly when dealing with adult sheep, does not bear an exact relationship to the numbers of gastro-intestinal parasites present in the alimentary tract, but nevertheless the above findings are of interest. They demonstrate that pining sheep do harbour internal parasites, a fact which hitherto has not been recorded, and since the anæmia varies with the egg index, it can no longer be doubted that alimentary parasites constitute at least one factor in producing the blood picture which characterises Northumbrian pining.

Farm B.—Situated on Cheviot range. The sheep walks are similar in appearance to those of Farm A, and the management of the flock is conducted under a similar system. This is likewise a reputed pining farm, and the stock is changed once in the spring and again in autumn. Pining was stated to be more serious among the lambs, making its appearance after weaning.

Three groups of five sheep were again selected as in Farm A, namely, a pining group Nos. 16 to 20, a non-pining group Nos. 21 to 25 and a second pining group Nos. 26 to 30. Table VI (Appendix) records the complete blood figures obtained from these ewes from the commencement of observation until the experiment was discontinued in March, 1934.

Reference to Table VI shows that, on the whole, the results obtained were very similar to those on Farm A. The increase in the blood elements in November and December is shown, together with their subsequent decline. The inverse relationship between the egg index and the blood constituents is marked in sheep Nos. 16, 21 and 23.

As on Farm A., a third group of five pining sheep was chosen (Group III, Table VI), but instead of iron, these ewes were given 18 grains of powdered bluestone, which was thrown on the back of the tongue. Treatment commenced on July 26th, and was continued weekly for eight weeks. During this period these ewes improved in condition, but as in the corresponding Group III on Farm A, this experiment was to a large extent nullified by the corresponding improvement in the two untreated groups.

Summary.

(1) On two affected farms typical cases of pining have been observed for (*a*) eighteen months and (*b*) eight months. The symptoms noted were those of anæmia and emaciation.

(2) Examination of the blood of typical cases showed a content of red cells and hæmoglobin approximately one-half that of normal sheep blood. The colour index and average cell volume were not materially altered, and the blood serum showed no evidence of hæmolysis.

(3) Differential leucocyte counts made on the blood of sheep on pining farms showed a decreased proportion of neutrophiles and an increased proportion of lymphocytes and eosinophile cells.

This corresponds to the picture previously recorded for sheep in well-marked infestations with gastro-intestinal parasites.

(4) Examination of the fæces for the ova of parasitic worms showed that gastro-intestinal parasites were present in all the cases of pining sheep examined. The egg index was observed to vary inversely with the severity of the disease and intensity of anæmia.

(5) In two experiments designed to test the curative effect of (*a*) iron and (*b*) copper salts, definite conclusions could not be drawn owing to the parallel improvement in the untreated control sheep. The opinion is advanced that as the year proceeded the general improvement noted was more in the nature of a normal seasonal variation in condition.

(6) A low figure for serum calcium was noted in pining and non-pining ewes on affected farms.

Pathological.

In Northumberland pining occurs as a disease of ewes and lambs, the critical period for the latter being the months of July and August, when there is frequently anxiety as to whether or not lambs can be maintained healthy until the August sales. May and June are the worst months for hill ewes, and many farmers change their ewes in March prior to lambing, while others prefer to change in June and again in September. The keynote of success is, of course, to change the stock before the appearance of symptoms, and it was generally stated that ewes visibly pining in June would die from the disease unless changed. It was anticipated, therefore, that by choosing pining ewes in June, and by withholding the customary change so as to keep them under observation on the affected farms, a complete picture of the progressive disease would be obtained, to be completed later by *post-mortem* examinations.

Reference to the tables, however, shows that this did not prove to be the rule, because of the 20 pining ewes on Farms A. and B. only nine died (or were killed *in extremis*) from pining in the period under review, four were sold in October, 1933, in fair condition, and seven were alive in December, 1934. *Post-mortem* examinations were carried out on eight pining ewes, and a brief account of the findings is given. In addition, five typical cases were obtained from two other pining farms, two in July and the other three in December when the acute effects of the disease had passed off. The results of these *post-mortems* are also briefly recorded.

Sheep No. 1.—Black-faced ewe, four years. Unable to rear her lamb, and on June 22nd was very weak and anæmic. Affected six weeks, showing progressive debility. Body emaciated, fleece hard and dry, eyes bright and no evidence of diarrhœa. Examination showed Egg Count 3,700. Red cells 4·85 mills. Red cell volume 15. Hb. 38. Shepherd stated that a complete change of pasture might possibly effect a cure, otherwise death was certain. Death occurred *en route* to laboratory.

Post-mortem.—23.6.33. The subcutaneous tissues anasarcous, pale in colour, and a litre of clear dropsical fluid was present in peritoneal cavity. The internal organs appeared blanched and shrunken. Abomasum contained 200 c.c. of liquid, green ingesta, together with sand, soil, etc., and mucous membrane showed thickened and roughened areas and patches of congestion. There were no hæmorrhages and no ulceration of the surface, but nematode parasites were observed clinging to mucous folds. Lymphatics œdematous. Small intestine contained half litre fluid ingesta, was bile-stained throughout its length and slightly inflamed. The liver was pale, small, but firm in texture and free from liver flukes or cirrhosis. No abnormality noted in the other internal organs. The heart was surrounded by about 100 c.c. of partially coagulated pericardial fluid, and its musculature was pale and flabby; no coronary fat. Right heart contained large antemortem clot. Many small hæmorrhages on the surfaces of the auricles.

The contents of the abomasum and small intestine were examined for parasites by washing and centrifuging.

Parasites present.—Abomasum approximately 60,000 parasites, mostly *O. circumcincta* and *H. contortus*. Intestines about 10 yards of tapeworms (*M. expansa*) and numerous *H. contortus* and *N. filicollis*. Number not counted. Sand and dirt also present. Scores of *C. ovina* found in latter part of gut.

Diagnosis.—This ewe was affected with parasitic gastro-enteritis, a heavy infestation, chiefly of *O. circumcincta*, *H. contortus*, *N. filicollis* and *C. ovina* being present. Tapeworms were also present.

Sheep No. 5.—Black-faced ewe, six years. Observed pining in June, but improved during summer and became weaker in November. Did not rear lamb and could not be clipped in July. In November, general condition poor. Examination 16.11.33 showed Egg Count 700. Red cells 8·43 mills. Red cell volume 26. Hb. 69. Marked neutrophile leucocytosis. Killed by chloroform 19.11.33.

Post-mortem.—Abdomen contained one litre peritoneal fluid. Little kidney and omental fat present. Contents of abomasum lightly scraped into beaker. Mucous membrane thickened and roughened due to activity of parasites, but no inflammation nor congestion. Surface throughout studded with small, raised plaque-like areas, resembling healed abrasions. Small intestine contained 750 c.c. ingesta. Tapeworms evident. No inflammation but patches of congestion. Smears made from mucosa of intestine and stained with Ziehl-Neelson failed to reveal presence of acid-fast micro-organisms. Other internal organs appeared normal, except heart which was flaccid and surrounded with pericardial fluid. Few parasitic nodules present in lung tissue.

Parasites present.—Abomasum mixed infestation of roundworms (not counted), *O. circumcincta*, *O. trifurcata* and *H. contortus* predominating. Intestine. Half cupful of tapeworms, also *C. curticei*, *N. filicollis* and *T. extenuatus*. Actual counts not made. These species of parasites confirmed by Dr. R. L. Leiper.

Diagnosis.—The *ante-mortem* and *post-mortem* appearances were those of a thin and weak animal suffering from parasitic gastritis.

Sheep No. 15.—Black-faced ewe, five years. Observed weak after lambing in April, 1933, and unable to rear lamb. Remained unclipped. Condition improved during summer, and in autumn was mated, later producing a weak lamb. Was weak and anæmic in July, 1934, and pouched

below the jaw. Could not be clipped and therefore carried three fleeces. Examination 21.6.34 showed Egg Count 1,600. Red cells 6·65 mills. Red cell volume 15. Hb. 45. Marked lymphocytosis. No evidence of diarrhœa.

Post-mortem.—27.7.34. Two litres of abdominal fluid present. Internal organs pale in appearance and shrunken. Surface of abomasum thickened and showed the typical grained leather-like appearance of parasitic gastritis. Stomach wall dropsical, lymphatics soft and œdematous. No inflammation of intestines and no congestion. Mucosa examined for acid-fast micro-organisms but none detected. About one litre of ingesta in abomasum. Other organs appeared normal, except heart, which was atonic and surrounded by fluid.

Parasites present.—Abomasum 38,600 worms, chiefly *O. circumcincta*, *H. contortus* and *C. oncophora*. Intestines 8,200 worms, chiefly *C. oncophora*, *H. contortus* and *M. expansa*. *T. ovis* and *C. ovina* present in large intestine.

Diagnosis.—Parasitic gastritis.

Sheep No. 12.—Black-faced ewe, four years. Slightly affected July, 1933, but improved till January, 1934. Reared poor lamb. Condition improved after weaning, but became weaker early winter. Emaciated in December, 1934. Examination 18.12.34 showed Egg Count 1,400. Red cells 7·06 mills. Red cell volume 18. Hb. 64. Marked lymphocytosis. No evidence of diarrhœa. Killed 19.12.34.

Post-mortem.—19.12.34. About one litre clear fibrinous fluid present in abdomen. Internal organs blanched and shrunken. Abomasum contained 200 c.c. ingesta, mucosa thickened, roughened and slightly congested near tip of folds. Sand and dirt present. Small intestine contained half litre ingesta ; gut bile-stained throughout, but no acid-fast micro-organisms detected. Liver small, pale, but firm in texture ; no liver flukes. All lymphatics dark-coloured and œdematous. Other organs appeared normal except heart, which was pale, flabby and surrounded by fibrinous fluid.

Parasites present.—Abomasum 5,700 worms, chiefly *O. circumcincta*, also *C. oncophora* and *H. contortus* (very few). Small intestine 4,000 worms, chiefly *C. oncophora*, *O. circumcincta* and *M. expansa*. Large intestine, *T. ovis* and *C. ovina*.

Diagnosis.—The history of Ewe 12, and the comparatively light worm burden disclosed *post-mortem*, preclude diagnosis of ordinary parasitic gastritis. Her final condition appeared due to under-nutrition, probably assisted or complicated by parasitic infestation.

On Farm B. four pining ewes died from the disease, and three were examined *post-mortem*, together with one non-pining control ewe, which subsequently developed pining. The other six pining ewes on this farm greatly improved in condition, and of this six it is of interest to note that four belonged to the group which received weekly doses of copper sulphate. A brief account of the *post-mortem* appearances of the ewes which died is appended.

Sheep No. 29.—Cheviot ewe, four years. Affected pining, June, 1933. Unable to rear her lamb and was not clipped in July. Appeared to improve, but died 1.8.33. Carcase emaciated. Examination 26.7.33. Egg Count 300. Red cells 6·17 mills. Red cell volume 22. Hb. 59. Slight neutrophile leucocytosis. Some diarrhœa. Brought to laboratory.

Post-mortem.—2.8.33. Abdominal fluid present, and internal organs pale, shrunken and œdematous. Abomasum contained 300 c.c. fluid ingesta ;

mucosa roughened and thickened, especially near edges of folds. Sand present. Small intestine bile-stained. No tapeworms. Apart from small size the other organs appeared normal, except heart which was flaccid and pale ; some fibrinous pericardial fluid.

Parasites present.—Animal severely parasiticised (number not counted), chiefly *O. circumcincta*, *O. trifurcata* and *N. filicollis*. *C. ovina* and *T. ovis* present in large intestine.

Diagnosis.—Parasitic gastritis.

Sheep No. 18.—Cheviot ewe, four years. First observed pining in July, 1933. Reared poor lamb. Examination 23.8.33 showed Egg Count 1,000. Red cells 4·78 mills. Red cell volume 14. Hb. 40. Slight lymphocytosis Total white cells 4,000. No diarrhœa. Carcase emaciated. Killed 24.8.33.

Post-mortem.—24.8.33. Abdominal fluid present, and internal organs pale and shrunken. Mucous membrane of abomasum thickened and leathery in appearance, wall and folds somewhat œdematous. Sand and dirt present. Lymphatics œdematous. About one litre fluid ingesta in intestine ; congested in patches. All other organs appeared normal except heart which was flaccid and surrounded by fluid.

Parasites present.—Abomasum 21,500 worms, chiefly *O. circumcincta*, *O. trifurcata*, *T. extenuatus*, *H. contortus*. Intestine 14,000 worms, chiefly *N. filicollis*, *C. curticei*, *T. colubriformis*, *T. vitrinus*. *C. ovina* were found in the large intestine. These species of worms were kindly identified by Dr. D. O. Morgan.

Diagnosis.—Parasitic gastro-enteritis.

Sheep No. 19.—Cheviot ewe, five years. Affected pining June, 1933. Continued unthrifty throughout summer, but improved December. Improvement not maintained, and was noted weak and coughing early in February, 1934. Examination 24.1.34 showed Egg Count 500. Red cells 10·53 mills. Red cell volume 27. Hb. 91. Slight neutrophile leucocytosis. No diarrhœa. Died 16.2.34. Brought to laboratory.

Post-mortem.—17.2.34. Peritoneal cavity contained approximately one gallon clear fluid. Thoracic cavity also contained fluid which was blood-stained. Internal organs shrunken, pale and œdematous. Abomasum contained two ounces semi-solid ingesta. Mucosa slightly thickened. Intestine showed catarrhal inflammatory changes ; contents scanty. No acid-fast micro-organisms detected. Lungs showed broncho-pneumonia with apical consolidation. Other organs appeared normal, except heart which was pale and flaccid.

Parasites present.—Abomasum 12,500 worms, chiefly *O. circumcincta* ; few *H. contortus*. Small intestine 2,400 chiefly *T. extenuatus*. *C. ovina* present in large gut. No lungworms.

Diagnosis.—Death apparently due to broncho-pneumonia, effusion and collapse. No. 19 was considered a typical piner, and it is possible that the final illness was associated with the undermining effect of gastro-intestinal parasites.

Sheep No. 21.—Cheviot ewe, five years. Considered healthy July, 1933, but except for temporary improvement in November, 1933, gradually became emaciated and died 6.3.34. Examination 26.2.34 showed Egg Count 5,100. Red cells 5·21 mills. Red cell volume 20. Hb. 48. Neutrophile leucocytosis. Some diarrhœa. Brought to laboratory.

Post-mortem.—7.3.34. Abdominal cavity contained two quarts blood-stained fluid ; no rupture detected. Carcase dropsical ; viscera pale and shrunken. Abomasum contained small amount of ingesta, also sand and dirt. Mucosa pale, œdematous, slightly inflamed and roughened. Typical grained, leather-like appearance noticeable in areas of abomasum. Small intestine showed chronic catarrhal condition, with patches of congestion. Bile-stained throughout ; no acid-fast micro-organisms detected. No tapeworms. Other organs appeared normal, except lungs, which showed broncho-pneumonia.

Parasites present.—Abomasum 10,500 worms. Chiefly *O. circumcincta*, few *H. contortus*. Intestine. *B. trigonocephalum*, *C. oncophora*, *N. filicollis*. Hundreds of *C. ovina* in large intestine. No lungworms.

Diagnosis.—In all probability death was due to broncho-pneumonia. The presence of parasitic gastro-enteritis may have been contributory.

In addition to the foregoing, two pining sheep were examined in July, 1934, and three ewes, stated to be suffering from the after-effects of pining, were also submitted to *post-mortem*, particulars of which are appended.

*Sheep A*1.—Cheviot ewe, two years. Was weak after lambing in April and unable to rear lamb ; by July was emaciated and remained unclipped. Examined 19.7.34. Anæmic, emaciated, dropsical but no diarrhœa, and was considered typical case of pining. Examination showed Egg Count 5,200. Red cells 7·5 mills. Red cell volume 21. Hb. 58. White cells 15,200. Neutrophile leucocytosis. Brought to laboratory. Died 19.7.34.

Post-mortem.—20.7.34. Dropsical fluid present in abdomen. Viscera pale and shrunken. Abomasum contained 200 c.c. fluid ; sand and dirt present. Mucosa showed typical grained, leather-like appearance associated with parasitic gastritis, marked near pyloric portion. The folds were inflamed and a dull livid colour ; also thickened in parts. Many small worms on mucosa. Intestine showed catarrhal inflammation. Lymphatics soft, dark in colour and œdematous. No acid-fast micro-organisms detected. Tapeworms present. The edges of lungs were solid and contained many parasites. Broncho-pneumonia present. Other organs appeared normal, except heart which was large, flabby and surrounded with pericardial fluid.

Parasites present.—Abomasum. 17,000. Chiefly *O. circumcincta*. Intestine. 26,000. Chiefly *C. oncophora*, *T. extenuatus*, *M. expansa*. *C. ovina* present in colon and rectum. Lungs. *D. filaria* and *M. capillaris*.

Diagnosis.—Parasitic gastro-enteritis and parasitic broncho-pneumonia.

*Sheep A*2.—Cheviot ewe, five years. Observed pining May, 1934, and was weak and emaciated in July. Examination 28.7.34 showed Egg Count 8,000. Red cells 5·6 mills. Hb. 52. Slight lymphocytosis. Some diarrhœa. Died 28.7.34.

Post-mortem.—28.7.34. Carcase dropsical and internal organs pale and shrunken. One litre fluid present in abdominal cavity. Abomasum contained about 400 c.c. contents. Mucosa thickened roughened and showed grained, leather-like appearance typical of parasitic gastritis. Slight catarrhal inflammatory change in intestines, which were bile-stained ; no acid-fast micro-organisms detected. Little broncho-pneumonia present. Other organs appeared normal except heart which was enlarged, pale and flabby.

Parasites present.—Abomasum 47,200 worms, chiefly *O. circumcincta*, *T. colubriformis* and few *H. contortus*. Intestine 15,300 worms, chiefly

N. filicollis and *C. oncophora*. Many *C. ovina* present in large gut. *D. filaria* present in lungs.

Diagnosis.—Parasitis gastro-enteritis, also parasitic broncho-pneumonia.

*Sheep A*3.—Cheviot ewe, three years. Observed pining in May, 1934, and continued unthrifty in spite of change of pasture in June ; remained unclipped. Brought from hill to meadow in October ; appeared to recover somewhat, but was in poor condition in December. Owner stated this ewe was a typical piner in May, and was now suffering from its after effects. Prolonged change to richer pastures would probably effect cure. Examination 16.12.34 showed Egg Count 1,300. Red cells 11·90 mills. Red cell volume 33. Hb. 102. White cells 9,400. Marked lymphocytosis (70 per cent.). Ca. 8·15 mgs. P. 11·68 mgs. No diarrhœa. Killed 18.12.34.

Post-mortem.—18.12.34.—Some clear fibrinous fluid in abdomen. Small quantity of fat in carcase. Abomasum contained 300 c.c. ingesta. Mucous membrane pinkish ; showed little thickening and roughening indicative of parasitic gastritis. At two points the membrane was puckered owing to contraction of healing scars. Lymphatics dark and œdematous. Small intestinal mucosa slightly congested and bile-stained ; no acid-fast micro-organisms detected. Some parasitic broncho-pneumonia. Other organs appeared normal.

Parasites present.—Abomasum 5,000 worms. *O. circumcincta*. Intestine 4,600, chiefly *Cooperia* species and *N. filicollis*. Many *T. ovis* and *C. ovina*. Lungs *D. filaria*.

Diagnosis.—This ewe was apparently suffering from under-nutrition, and showed evidence of slight parasitic gastritis and broncho-pneumonia.

*Sheep A*4.—Cheviot ewe, six years. Observed pining in May, 1934, and remained unthrifty throughout summer, despite the usual change ; unable to be clipped. In October was taken from hill and placed in meadow, when condition visibly improved. Owner considered she would recover on richer pasture, failing which death was certain. She was regarded not so much a piner, as suffering from the after-effects of pining. Examination 16.12.34 showed Egg Count 8,200. Red cells 12·92 mills. Red cell volume 36. Hb. 108. White cells 7,600. Marked lymphocytosis (73 per cent.). Ca. 5·07 mgs. P. 11·57 mgs. No diarrhœa. Killed 18.12.34.

Post-mortem.—18.12.34. Fibrinous fluid present in abdomen ; some kidney fat. Abomasum was thickened and roughened, showing grained leather-like appearance indicative of parasitic gastritis. Intestine bile-stained and showed catarrhal exudate ; no acid-fast micro-organisms detected. Other organs appeared normal.

Parasites present.—Abomasum 6,900 worms. Chiefly *O. circumcincta*. Intestine 900. *C. oncophora* and *N. filicollis*. *T. ovis* and *C. ovina* present in large intestine.

Diagnosis.—This ewe was apparently suffering from under-nutrition, and showed evidence of slight parasitic gastro-enteritis.

*Sheep A*5.—Cheviot ewe, six years. Observed pining in June, 1934, and remained unthrifty throughout summer despite change of pasture ; unable to be clipped. Condition poor in December, when considered suffering from after-effects of pining. Examination 16.12.34 showed Egg Count 4,700. Red cells 9·16 mills. Red cell volume 28·5. Hb. 80. White cells 10,400. Slight neutrophile leucocytosis (50 per cent.). Ca. 7·96 mgs. P. 5·84 mgs. No diarrhœa. Killed 18.12.34.

Post-mortem.—18.12.34.—Similar to that of Sheep A4, but molar teeth were large, sharp and irregular, no doubt interfering with mastication. Evidence of parasitic gastro-enteritis. Other organs appeared normal. No acid-fast micro-organisms detected.

Parasites present.—Abomasum 3,900 worms. *O. circumcincta.* Intestine 2,200, chiefly *C. oncophora. T. ovis* and *C. ovina* present in large intestine.

Diagnosis.—This ewe appeared to be suffering from under-nutrition. Defective molar teeth possibly contributory. Also evidence of slight parasitic gastro-enteritis.

Summary.

(1) Records are presented of *ante-mortem* and *post-mortem* observations on eight pining sheep which have been observed on affected farms for periods up to 18 months. Two other pining ewes from affected farms were examined when at the height of the disease. In addition, *post-mortem* examinations were conducted on three ewes stated to be suffering from the after-effects of pining, and these records are included.

(2) Nematode parasites belonging chiefly to the subfamily trichostrongylinæ were present in the abomasum and intestines of all cases examined. The numbers found varied between 60,000 and 6,000, the largest numbers being present in sheep examined during the summer months, when pining is at its height.

(3) In all 13 cases there was damage to the mucosa of the abomasum, the typical lesion consisting of a thickening and roughening of the membrane, prominent on the folds and also in the plain pyloric portion. This thickening resembles the appearance of grained leather.

(4) A straight pathological diagnosis of parasitic gastritis, with or without enteritis in addition, has been recorded in seven of the 13 cases. Of these, all but one case were examined after death during the summer months, which is the season when the natural disease manifests itself.

(6) From the history of the above six cases it appears probable that sheep which survive a summer attack of pining remain unthrifty, and frequently die during the following winter. With the onset of harder winter conditions they are unable to obtain sufficient nourishment, become weak, and death may occur from various end causes, such as broncho-pneumonia (Sheep Nos. 19 and 21). At the time of death their burden of parasites may be light.

(7) In all cases in which the mucosa of the small intestine was examined for acid-fast micro-organisms these were found to be absent.

Discussion

The disease known as " pining " as observed in Northumbrian hill sheep is defined, and brief reference has been made to earlier writings on the subject. Later views on pining are noted, particularly the modern tendency to co-relate pining with the

so-called deficiency diseases occurring in different parts of the world, such as " bush sickness " in New Zealand, " Nakuruitis " in Kenya, " salt sick " in Florida, " pine " in Tiree, etc. Mention has also been made of two diseases of sheep which simulate pining, one of which occurs in Northumbrian valleys adjacent to the pining areas, and is known as " blood rot." The senior author has examined 14 cases of blood rot and has come to the conclusion that the condition is acute hæmonchosis. Blood rot may be distinguished clinically from pining by its more rapid onset, course and termination; in other respects the symptoms of these two diseases are similar, if not identical. Finally, the opinion is offered that in the absence of pathological data, blood and hæmoglobin determinations and other observations on pining sheep, it is unjustifiable to link pining with the so-called deficiency diseases, and the present article records an attempt to elicit more precise information on the pathology of pining.

Data are presented from groups of pining and non-pining sheep, which were observed on affected farms for periods up to 18 months. The prominent symptoms in pining are anæmia and emaciation, the blood picture in typical cases showing a reduction in red cells and hæmoglobin to approximately half normal values. No hæmolysis in the serum was noted, and the colour index in pining sheep did not appear materially altered. Fourie[26] examined the blood picture in artificially produced hæmonchosis in sheep, and found the anæmia produced to be hæmatologically identical with that produced by bleeding sheep daily from the jugular vein. He also found that the colour index rose during the course of the disease, due to increase in the size of the red cells, and noted morphological changes, *i.e.*, anisocytosis, poikilocytosis and punctate basophilic cells. In the present series of cases these changes were only rarely observed in the blood from pining sheep, and figures for the average individual cell volumes do not indicate changes in the size of the red corpuscles. It must therefore be concluded that the anæmia in pining is not identical with that described by Fourie in hæmonchosis, and that the decrease in hæmoglobin and red corpuscles occurs *pari passu,* so that the colour index did not show any regular or significant alteration.

The differential leucocyte counts carried out on the blood of sheep on pining farms showed a decreased proportion of neutrophiles and an increased proportion of lymphocytes and eosinophile cells. These altered proportions correspond to changes in the white blood corpuscles previously recorded in well-marked infestations with gastro-intestinal parasites, and fæcal examinations disclosed the presence of ova of trichostrongyle worms in the majority of sheep examined. Further, the number of ova in the fæces was observed to vary inversely with the severity of the disease and intensity of anæmia.

Post-mortem examinations were carried out on 13 pining sheep and evidence of parasitic gastritis was noted in every case. The mucosa of the abomasum in all cases showed a characteristic thickening and roughening which is absent from the stomachs of healthy sheep; for example, those examined at the abattoir. The thickening affects the folded and more regular pyloric portion of the abomasum and has been likened to the appearance of grained leather. Parasites of the genus Ostertagia were found predominating throughout the series of cases and the characteristic thickening probably results from the burrowing habits of the immature worms. Dikmans and Andrews[27] examined the fourth stomachs of sheep after they had grazed on infected pastures for three weeks, and found the larvæ of Ostertagia in small, raised, white areas of the mucous membrane. Dark coloured spots near the pylorus were found to contain nearly mature specimens of *O. circumcincta*, and these authors are of opinion that the early forms of this parasite live in the mucosa and do not emerge until almost adult. Threlkeld[28] has likewise investigated the life history of *O. circumcincta* and noted the burrowing habits of the infective larvæ, which he states are coiled deeply within the mucosa.

The numbers of parasites found in the abomasum and intestines of pining sheep were observed to vary with the time of year when the *post-mortem* was conducted, the largest numbers being obtained from cases examined during the summer months. In these cases it was possible to diagnose parasitic gastritis and enteritis, but in the cases which survived until winter or early spring there was a reduced total parasite count, together with complications such as under-nutrition and broncho-pneumonia. The writers feel that it can no longer be doubted that many cases of Northumbrian pining are really cases of parasitic gastro-enteritis, and the experimental evidence here provided supports this view. As seen by the writers, Northumberland pining bears a close resemblance to blood rot, which has already been shown to be parasitic gastritis (*H. contortus* predominating), the chief points of difference being (*a*) in blood rot there is a more rapid onset and termination, so that (*b*) blood rot is rarely seen except in June and July, when it is common in certain districts. While pining is at its height during the summer months, subacute or chronic cases frequently survive the autumn and winter months and some linger until early spring when death may occur from a variety of end causes. This prolonged unthriftiness is sometimes taken as evidence that the trouble is primarily a nutritional disorder. How much of this interference with nutrition results from the presence of internal parasites and the burrowing habits of certain immature forms, cannot be stated with precision, but it is planned to deal more fully with this aspect of the problem in a subsequent article.

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TABLE I.

INITIAL BLOOD ANALYSES. GROUPS I, II AND III.

	No.	Egg Index 100s. per gm.	Vol. % Red Cells.	Av. Indiv. Cell Vol.	Red Blood Cells per c.mm.	Hæmoglobin gm. %	Colour Index.	Hæmoglobin Sh. Sc.	White Blood Cells.	Ser. Ca.	Blood Inor. Phos.
June 7—											
Group I	1	13	18	2·82	6·38	6·05	0·95	53	8,200	8·4	6·12
Pining	2	9	13	2·60	5·00	5·10	1·02	45	2,000	8·5	4·02
	3	14	20	2·76	7·24	7·40	1·02	65	5,400	8·1	4·35
	4	25	18	2·97	6·07	7·68	1·26	67	3,700	8·2	4·19
	5	22	15	3·61	4·16	4·37	1·05	38	4,400	8·6	6·19
Average ...		17	17	2·95	5·77	6·12	1·06	54	4,740	8·3	4·97
June 7—											
Group II ...	6	1	30	2·90	10·35	11·10	1·07	97	7,600	9·2	4·09
Non-Pining	7	0	32	2·84	11·28	11·60	1·03	101	9,200	9·0	5·49
	8	0	30	2·93	10·24	12·25	1·22	107	6,600	9·9	6·68
	9	2	31	2·78	11·15	10·20	0·92	89	6,200	9·0	4·89
	10	0	28	2·92	9·60	12·00	1·25	105	6,300	8·9	5·61
Average		1	30	2·87	10·52	11·43	1·10	100	7,180	9·2	5·35
July 6—											
Group III	11	1	30	2·93	10·24	10·40	1·02	91	—	11·5	4·62
Pining ...	12	0	30	2·91	10·31	9·80	0·95	86	8,800	11·3	4·27
Iron Treatment	13	5	20	3·03	6·61	5·99	0·91	52	7,000	10·7	4·41
	14	7	27	3·24	8·34	8·68	1·04	76	7,000	11·7	4·12
	15	16	17	2·83	6·00	5·41	0·90	47	6,400	10·5	4·23
Average ...		6	25	2·99	8·30	8·05	0·97	70	7,300	11·1	4·33

TABLE II.

SHOWING DIFFERENTIAL LEUCOCYTE COUNTS ON PINING AND HEALTHY SHEEP ON FARM A.

	No.	Total Whites.	Neutrophiles.	Lymphocytes.	Large Monocytes.	Eosinophiles.	Basophiles.
June 22—							
Group I							
Pining	1	11,800	48	46	5	1	0
	2	4,100	24	66	8	2	0
	3	6,600	35	44	10	11	0
	4	6,000	20	42	6	28	4
	5	5,400	16	66	9	3	6
Average		6,780	29	53	8	9	2
Group II...							
Non-Pining	6	6,600	27	61	7	5	0
	7	6,800	23	54	4	19	0
	8	9,400	15	77	2	6	0
	9	10,000	23	54	4	19	0
	10	8,800	28	60	2	10	0
Average		8,320	23	61	4	12	0
Fraser's Normal Sheep		8,000	39	52	3·2	4·9	0·7

TABLE III.

COMPLETE BLOOD PICTURES. GROUP I. PINERS.

	Egg Index 100s. per gm.	Vol. % Red Cells.	Av. Indiv. Cell Vol.	Red Blood Cells per c.mm. Mills.	Hæmoglobin gm. %	Colour Index.	Hæmoglobin Sh. Sc.	Neutrophiles. %	Lymphocytes. %	Large Monocytes. %	Eosinophiles. %	Basophiles. %	Total White Blood Cells per c.mm.	Ser. Ca.	Blood Inorg. Phos.
Sheep I—															
June 7	13	18	2·82	6·38	6·05	0·95	53	—	—	—	—	—	8,200	8·4	6·12
„ 22	37	15	3·09	4·85	4·40	0·91	39	48	46	5	1	0	11,800	8·9	3·95
Died 22.6.33															
Sheep II—															
June 7	9	15	3·00	5·00	5·10	1·02	45	—	—	—	—	—	2,000	8·5	4·02
„ 22	18	13	2·63	4·95	5·70	1·15	50	35	44	10	11	0	4,100	8·8	3·90
July 6	17	15	2·77	5·41	5·22	0·96	46	—	—	—	—	—	2,400	10·4	3·69
Oct. 19	0	33	2·78	11·87	11·10	0·94	97	28	49	3	19	0	5,200	11·3	5·92
Nov. 16	0	34	3·37	10·10	9·20	0·91	80	34	50	2	14	0	7,400	—	—
Dec. 19	1	32	2·85	11·22	9·90	0·89	86	20	58	2	20	0	5,200	10·3	6·43
Jan. 15	2	30	2·60	11·52	10·00	0·87	87	35	48	2	13	2	6,600	11·3	5·92
Feb. 12	0	32	3·04	10·53	9·70	0·92	85	34	54	2	9	1	3,200	—	—
Mar. 27	0	31	3·09	10·03	10·20	1·02	89	28	53	2	17	0	4,800	9·3	4·02
May 8	2	33	3·36	9·81	10·20	1·04	89	25	64	2	9	0	3,600	9·9	7·11
June 21	16	28	3·44	8·15	9·40	1·15	82	35	51	3	10	1	3,600	—	—
Sept. 17	4	29	2·48	11·71	11·30	0·96	99	27	55	1	17	0	6,000	10·6	3·90
Sheep III															
June 7	14	20	2·76	7·24	7·40	1·02	65	—	—	—	—	—	5,400	8·1	4·35
„ 22	10	18	2·76	6·52	5·70	0·87	49	24	66	8	2	0	6,600	9·8	4·00
July 6	11	20	2·81	7·11	6·53	0·92	57	—	—	—	—	—	6,800	10·6	4·00
„ 17	23	21	2·84	7·40	6·51	0·88	57	38	56	2	2	2	10,400	10·2	4·00
Aug. 21	15	30	2·86	10·50	9·80	0·93	86	—	—	—	—	—	6,800	—	—
Oct. 19	0	35	2·83	12·39	11·50	0·93	100	24	69	2	5	0	6,200	10·4	6·14
Nov. 16	1	35	3·18	11·00	9·60	0·87	84	22	68	3	6	1	5,400	—	—
Dec. 19	0	32	2·83	11·31	9·70	0·86	85	31	66	2	3	0	8,200	9·4	6·75
Jan. 15	1	34	2·57	13·22	10·90	0·83	95	26	66	2	6	0	5,600	9·7	6·00
Feb. 12	0	35	3·11	11·26	10·20	0·91	89	22	66	1	11	0	5,800	—	—
Mar. 27	0	29	2·64	11·00	10·70	0·97	93	30	53	2	15	0	6,000	8·1	5·15
May 8	1	40	3·38	11·83	12·00	1·01	105	34	60	11	4	0	6,000	9·9	5·67
June 21	12	24	2·97	8·08	8·08	1·00	71	50	41	1	8	0	6,400	—	—
Sept. 17	48	25	2·85	8·76	9·30	1·13	81	84	15	1	0	0	14,600	9·4	5·50
Oct. 30	14	25	3·01	8·32	7·70	0·93	67	31	56	—	13	Odd B.	7,600	7·5	—
Dec. 17	0	28	3·41	8·20	9·90	1·21	87	38	60	—	2	—	12,600	9·9	6·52

TABLE III (*contd.*).

	Egg Index 100s. per gm.	*Vol. % Red Cells.*	*Av. Indiv. Cell Vol.*	*Red Blood Cells per c.mm. Mills.*	*Hæmoglobin gm. %*	*Colour Index.*	*Hæmoglobin Sh. Sc.*	*Neutrophiles. %*	*Lymphocytes. %*	*Large Monocytes. %*	*Eosinophiles. %*	*Basophiles. %*	*Total White Blood Cells per c.mm.*	*Ser. Ca.*	*Blood Inorg. Phos.*
Sheep IV—															
June 7	25	18	2·97	6·07	7·68	1·26	67	—	—	—	—	—	3,700	8·2	4·19
„ 22	2	20	2·41	8·30	7·11	0·85	62	20	42	6	28	4	6,000	8·2	4·30
July 6	3	24	3·38	7·10	7·88	1·11	69	—	—	—	—	—	5,600	9·8	3·79
„ 17	—	25	3·11	8·04	7·31	0·91	64	28	62	2	7	1	9,600	8·2	4·50
Aug. 21	13	28	3·11	9·01	8·53	0·95	74	—	—	—	—	—	10,400	—	—
Oct. 19	—	28	2·81	9·98	9·60	0·96	84	14	72	2	12	0	4,800	10·3	5·82
Nov. 16	1	28	2·79	10·04	8·25	0·82	72	21	67	2	8	2	9,000	—	—
Dec. 19	0	31	2·87	10·79	9·20	0·85	80	8	73	2	17	0	11,200	10·1	6·25
Jan. 15	4	28	2·81	9·98	9·60	0·96	84	19	70	2	9	0	7,400	9·8	5·92
Feb. 12	0	30	2·62	11·43	9·10	0·80	80	15	74	1	10	0	7,400	—	—
Mar. 27	0	29	2·79	10·39	10·10	0·97	88	24	60	2	14	0	5,800	9·1	4·47
May 8	1	35	3·48	10·05	10·20	1·01	89	31	58	1	9	0	8,800	9·2	5·40
June 21	4	29	3·10	9·36	9·60	1·03	84	20	69	1	10	0	7,800	—	—
Sept. 17	2	29	2·59	11·18	11·10	0·99	97	21	70	1	8	0	8,600	9·0	5·20
Oct. 30	4	—	—	—	—	—	—	16	73	1	10	Odd B.	—	—	—
Dec. 17	0	31	2·80	11·06	11·40	1·03	100	19	67	—	14	—	6,400	7·9	6·59
Sheep V—															
June 7	22	15	3·61	4·16	4·37	1·05	38	—	—	—	—	—	4,400	8·6	6·19
„ 22	4	14	3·54	3·96	4·30	1·09	38	16	66	9	3	6	5,400	8·3	5·11
July 6	4	12	4·67	2·57	3·00	1·17	26	—	—	—	—	—	4,000	8·4	4·62
„ 17	5	17	3·71	4·58	5·40	1·17	47	28	42	1	28	1	6,600	8·9	3·61
Aug. 21	12	29	3·37	8·60	8·53	0·99	74	—	—	—	—	—	7,800	—	—
Oct. 19	8	27	3·15	8·58	8·19	0·96	72	27	46	2	25	0	10,400	9·2	4·50
Nov. 16	7	26	3·08	8·43	8·00	0·95	70	64	23	3	10	0	—	—	4·44

Killed for *post-mortem* examination—Nov. 1933 approx.

TABLE IV.

COMPLETE BLOOD PICTURES. GROUP II. NON-PINERS.

	Egg Index 100s. per gm.	Vol. % Red Cells.	Av. Indiv. Cell Vol.	Red Blood Cells per c.mm. Mills.	Hæmoglobin gm. %	Colour Index.	Hæmoglobin Sh. Sc.	Neutrophiles. %	Lymphocytes. %	Large Monocytes. %	Eosinophiles. %	Basophiles. %	Total White Blood Cells per c.mm.	Ser. Ca.	Blood Inorg. Phos.
Sheep VI—															
June 7	1	30	2·90	10·35	11·10	1·07	97	—	—	—	—	—	7,600	9·2	4·09
„ 22	1	30	2·83	10·59	10·20	0·96	89	27	61	7	5	0	6,600	9·2	4·96
July 6	2	30	2·93	10·27	10·40	1·01	91	—	—	—	—	—	11,000	10·2	4·50
„ 17	1	30	2·95	10·18	10·30	1·02	90	33	60	1	6	0	8,000	10·8	4·69
Aug. 21	0	32	3·25	9·85	10·20	1·04	89	18	60	2	19	1	7,800	—	—
Nov. 16	1	37	3·70	10·00	9·80	0·98	86	—	—	—	—	—	8,200	—	—
Dec. 19	3	31	2·95	10·50	9·10	0·89	79	18	70	2	10	0	12,800	10·4	7·50
Jan. 15	1	28	3·70	7·57	8·48	1·12	74	42	48	2	8	0	11,200	10·3	6·03
Feb. 12	0	33	3·38	9·76	9·00	0·92	79	18	60	2	20	0	9,200	—	—
Mar. 27	0	30	3·50	8·58	9·80	1·14	86	29	54	3	14	0	7,800	9·5	6·08
May 8	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
June 21	6	25	3·07	8·15	9·20	1·13	80	50	45	1	4	0	8,400	—	—
Sept. 17	4	28	3·24	8·64	8·80	1·02	77	28	53	1	16	0	13,600	9·7	6·30
Oct. 30	4	33	3·32	9·94	10·30	1·04	90	23	64	1	12	—	10,200	8·4	6·45
Dec. 7	6	32	3·46	11·62	11·80	1·02	103	16	72	—	12	—	7,600	8·8	8·04
Sheep VII—															
June 7	0	32	2·84	11·28	11·60	1·03	101	—	—	—	—	—	9,200	9·0	5·49
„ 22	0	32	3·16	10·12	9·80	0·97	86	23	54	4	19	0	6,800	9·5	4·79
July 6	0	32	2·81	11·39	10·60	0·94	93	—	—	—	—	—	10,600	11·4	4·30
„ 17	0	32	2·90	11·05	10·00	0·90	87	30	58	1	11	0	9,200	10·2	5·77
Aug. 21	9	33	2·78	11·89	11·10	0·93	97	9	86	2	3	0	11,800	—	—
Nov. 16	0	35	2·65	13·20	12·70	0·96	111	—	—	—	—	—	15,600	—	—
Dec. 19	0	32	2·60	12·32	10·60	0·86	93	25	58	2	15	0	7,000	11·2	8·44
Jan. 15	0	37	4·01	9·22	9·50	1·03	83	19	73	2	6	0	8,000	10·5	6·43
Feb. 12	0	33	3·41	9·67	10·70	1·11	93	8	82	2	8	0	10,800	—	—
Mar. 27	3	30	3·58	8·37	9·40	1·12	82	18	74	2	6	0	10,200	9·1	5·23
May 8	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
June 21	2	33	3·74	8·83	10·40	1·18	91	20	73	1	6	0	11,200	—	—
Sept. 17	4	30	4·03	7·45	7·32	0·99	64	33	65	1	1	0	7,600	10·8	7·80
Oct. 30	0	36	3·96	9·09	11·00	1·21	96	11	84	—	5	Odd B.	6,800	8·9	7·66
Dec. 17	4	30	3·25	9·39	10·60	1·13	93	43	52	1	4	0	9,400	8·2	5·98

TABLE IV. (*contd.*).

	Egg Index 100s. per gm.	*Vol. % Red Cells.*	*Av. Indiv. Cell Vol.*	*Red Blood Cells per c.mm. Mills.*	*Hæmo-globin gm. %*	*Colour Index.*	*Hæmo-globin Sh. Sc.*	*Neutro-philes. %*	*Lympho-cytes. %*	*Large Mono-cytes. %*	*Eosino-philes. %*	*Baso-philes. %*	*Total White Blood Cells per c.mm.*	*Ser. Ca.*	*Blood Inorg. Phos.*
Sheep VIII															
June 7	0	30	2·93	10·24	12·25	1·22	107	—	—	—	—	—	6,600	9·9	6·68
„ 22	0	29	2·89	10·05	9·60	0·96	84	15	77	2	6	0	9,400	10·2	5·00
July 6	0	34	3·00	11·33	10·60	0·94	93	—	—	—	—	—	7,400	11·4	4·47
„ 17	0	31	2·96	10·46	11·20	1·07	98	16	79	2	3	0	12,800	11·3	5·36
Nov. 16	1	31	3·03	10·23	10·46	1·02	91	16	75	2	7	0	10,600	—	—
Dec. 19	1	32	2·77	11·57	10·20	0·88	89	17	66	1	16	0	7,400	10·2	6·73
Jan. 15	0	33	2·99	11·03	11·40	1·03	100	23	64	2	11	0	9,400	10·6	5·15
Feb. 12	0	39	3·23	12·06	11·00	0·91	96	26	61	2	9	2	9,600	—	—
Mar. 27	4	32	3·08	10·40	10·20	0·98	89	34	59	2	5	0	6,800	9·1	4·50
May 8	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
June 21	0	37	3·22	11·48	11·10	0·97	97	37	54	1	8	0	7,600	—	—
Sept. 17	—	32	2·81	11·37	10·40	0·91	91	47	51	1	1	0	10,400	8·3	4·00
Sheep IX—															
June 7	2	31	2·78	11·15	10·20	0·92	89	—	—	—	—	—	6,200	9·0	4·89
„ 22	1	32	2·76	11·59	9·80	0·84	86	28	60	2	10	0	10,000	9·5	4·27
July 6	1	28	2·85	9·81	9·70	0·99	85	—	—	—	—	—	10,200	9·6	4·00
„ 17	1	32	2·81	11·37	10·70	0·94	93	31	56	3	9	1	8,800	8·9	3·95
Aug. 21	2	33	2·79	11·83	11·30	0·96	99	—	—	—	—	—	11,000	—	—
Nov. 16	1	34	2·84	11·96	11·40	0·95	100	28	59	3	9	1	—	—	—
Dec. 19	1	37	3·08	12·03	11·20	0·93	98	25	63	2	10	0	16,200	9·5	4·27
Jan. 15	2	36	3·09	11·65	11·30	0·97	99	27	53	2	18	0	13,000	9·8	4·66
Feb. 12	1	35	3·10	11·28	11·70	1·04	102	17	61	1	21	0	9,000	—	—
Mar. 27	2	32	2·81	11·39	11·60	1·02	101	24	60	1	15	0	11,200	10·3	5·77
May 8	0	34	3·25	10·46	10·40	1·00	91	37	36	2	25	0	8,800	9·5	7·42
June 21	2	30	3·56	8·43	10·00	1·19	87	31	45	1	23	0	10,000	—	—
Sept. 17	0	35	3·27	10·74	9·80	0·91	86	40	49	1	0	0	7,600	11·2	4·40
Oct. 30	0	35	3·62	12·42	12·80	1·03	112	17	66	1	16	Odd B.	9,600	8·7	5·90
Dec. 17	6	38	3·02	12·78	14·50	1·14	127	14	50	1	35	0	8,000	9·2	5·73

TABLE IV—(*Contd.*).

	Egg Index 100s. per gm.	*Vol. % Red Cells.*	*Av. Indiv. Cell Vol.*	*Red Blood Cells per c.mm. Mills.*	*Hæmo-globin gm. %*	*Colour Index.*	*Hæmo-globin Sh. Sc.*	*Neutro-philes. %*	*Lympho-cytes. %*	*Large Mono-cytes. %*	*Eosino-philes. %*	*Baso-philes. %*	*Total White Blood Cells per c.mm.*	*Ser. Ca.*	*Blood Inorg. Phos.*
Sheep X—															
June 7	0	28	2·92	9·60	12·00	1·25	105	—	—	—	—	—	6,300	8·9	5·61
„ 22	0	32	2·99	10·69	9·80	0·92	86	12	75	3	10	0	11,600	9·2	4·33
July 6	0	29	—	—	10·80	—	94	—	—	—	—	—	—	11·5	4·17
„ 17	0	31	2·67	11·62	11·30	0·97	99	22	62	4	12	0	10,800	9·8	4·35
Aug. 21	1	35	2·59	13·52	13·00	0·96	94	—	—	—	—	—	13,400	—	—
Nov. 16	1	32	2·67	12·00	11·52	0·96	101	21	63	3	13	0	—	—	—
Dec. 19	0	32	2·63	12·19	9·70	0·80	85	19	67	2	12	0	11,400	10·2	5·11
Jan. 15	1	33	3·33	9·91	10·50	1·06	92	48	42	2	7	1	10,400	10·8	4·50
Feb. 12	0	32	3·29	9·72	10·00	1·03	87	26	59	2	13	0	9,000	—	—
Mar. 27	2	31	3·41	9·10	10·20	1·12	89	40	50	2	8	Odd B.	6,600	9·1	4·44
May 8	0	29	4·59	6·32	8·00	1·27	70	43	46	2	9	0	8,000	10·0	8·04
June 21	0	33	3·12	10·59	11·00	1·04	96	34	56	1	9	0	8,200	—	—
Sept. 17	6	33	2·97	11·11	9·60	0·86	84	50	43	2	5	0	10,400	10·1	5·50

TABLE V.

COMPLETE BLOOD PICTURES. GROUP III.

Iron Treatment for two months commencing July 17th.

	Egg Index 100s. per gm.	*Vol. % Red Cells.*	*Av. Indiv. Cell Vol.*	*Red Blood Cells per c.mm. Mills.*	*Hæmoglobin gm. %*	*Colour Index.*	*Hæmoglobin Sh. Sc.*	*Neutrophiles. %*	*Lymphocytes. %*	*Large Monocytes. %*	*Eosinophiles. %*	*Basophiles. %*	*Total White Blood Cells per c.mm.*	*Ser. Ca.*	*Blood Inorg. Phos.*
Sheep XI—															
July 6	1	30	2·93	10·24	10·40	1·02	91	7	75	2	16	0	—	11·5	4·62
„ 17	3	35	3·16	11·09	10·50	0·95	92	25	70	3	1	1	10,000	10·2	3·20
Aug. 21	13	36	3·01	11·96	11·60	0·97	101	—	—	—	—	—	9,600	—	—
Oct. 19	0	—	—	—	—	—	—	—	—	—	—	—	—	10·3	5·11
Nov. 16	1	37	2·98	12·40	9·90	0·80	86	37	56	2	5	0	10,600	—	—
Dec. 19	1	38	2·85	13·34	10·60	0·79	93	18	73	2	6	1	14,200	9·7	7·50
Jan. 15	4	40	3·13	12·80	11·00	0·86	96	25	67	1	7	0	13,200	9·3	6·44
Feb. 12	0	40	3·41	11·73	11·50	0·98	100	16	70	2	12	0	8,200	—	—
Mar. 27	4	38	3·68	10·32	12·20	1·18	107	16	73	2	6	3	7,600	9·0	5·87
May 8	0	41	3·93	10·42	12·40	1·19	108	21	72	2	5	0	7,400	9·8	6·03
June 21	6	40	3·18	12·58	12·50	1·00	109	23	65	2	10	0	10,200	—	—
Sept. 17	12	37	2·94	12·62	10·30	0·82	90	21	70	1	8	0	10,400	8·9	8·00
Oct. 30	0	—	—	—	—	—	—	20	73	1	6	—	—	7·7	—
Dec. 17	6	39	2·60	15·22	13·30	0·87	116	19	75	2	4	—	4,800	8·6	5·95
Sheep XII—															
July 6	0	30	2·91	10·31	9·80	0·95	86	21	75	3	1	0	8,800	11·3	4·27
„ 17	0	31	3·08	10·10	8·94	0·89	78	24	70	4	1	1	10,800	11·3	4·66
Aug. 21	1	31	3·01	10·30	10·00	0·97	87	—	—	—	—	—	9,400	—	—
Oct. 19	0	28	2·92	9·58	8·80	0·92	77	16	78	2	4	0	10,600	10·6	6·89
Nov. 16	1	30	2·83	10·62	8·83	0·83	77	24	65	2	9	0	12,400	—	—
Dec. 19	0	33	2·91	11·35	10·00	0·88	87	11	83	2	4	0	14,800	8·6	8·23
Jan. 15	2	30	2·88	10·43	9·10	0·87	79	11	82	2	5	0	10,600	9·8	7·26
Feb. 12	0	28	3·02	9·26	9·30	1·00	81	12	81	2	5	0	8,600	—	—
Mar. 27	2	27	3·07	8·80	8·83	1·00	77	14	83	1	2	0	9,000	9·6	7·58
May 8	3	36	3·49	10·32	10·60	1·03	93	19	75	1	5	0	8,600	10·1	9·64
June 21	0	29	2·85	10·19	9·50	0·93	83	40	46	2	12	0	8,400	—	—
Sept. 17	4	32	2·53	12·67	11·30	0·89	99	29	67	1	3	0	14,400	10·5	7·10
Oct. 30	0	33	2·94	11·22	10·30	0·92	90	11	83	2	4	Odd B.	9,800	8·0	6·72
Dec. 18	14	20	2·83	7·06	7·40	1·05	65	12	85	—	3	—	5,200	8·4	5·07

TABLE V—(*Contd.*).

	Egg Index 100s. per gm.	*Vol. % Red Cells.*	*Av. Indiv. Cell Vol.*	*Red Blood Cells per c.mm. Mills.*	*Hæmo-globin gm. %*	*Colour Index.*	*Hæmo-globin Sh. Sc.*	*Neutro-philes. %*	*Lympho-cytes. %*	*Large Mono-cytes. %*	*Eosino-philes. %*	*Baso-philes. %*	*Total White Blood Cells per c.mm.*	*Ser. Ca.*	*Blood Inorg. Phos.*
Sheep XIII—															
July 6	5	20	3·03	6·61	5·99	0·91	52	—	—	—	—	—	7,000	10·7	4·41
„ 17	9	18	3·00	6·01	5·60	0·93	49	32	58	5	3	1	5,000	8·8	5·15
Aug. 21	8	30	3·34	8·98	9·20	1·02	80	—	—	—	—	—	8,200	—	—
Oct. 19	0	32	3·11	10·29	9·70	0·94	85	14	79	2	5	0	6,600	9·8	6·03
Nov. 16	5	35	2·99	11·69	10·60	0·91	93	39	55	2	4	0	11,800	8·5	7·50
Dec. 19	2	31	2·87	10·82	9·90	0·91	86	25	69	2	4	0	6,200	—	—
Jan. 15	1	31	3·13	9·92	10·00	1·01	87	21	71	2	6	0	8,600	9·6	6·43
Feb. 12	0	33	3·21	10·29	9·70	0·94	85	21	69	2	8	0	6,600	—	—
Mar. 27	1	32	2·98	10·73	10·10	0·94	88	14	80	2	4	0	7,200	8·8	7·26
June 21	2	37	3·37	10·97	11·40	1·04	100	41	49	2	8	0	8,000	—	—
Sept. 17	2	35	3·43	10·20	9·40	0·92	82	41	56	1	2	0	7,000	9·8	6·20
Oct. 30	2	36	3·03	11·89	11·00	0·93	96	20	73	2	5	Odd B.	6,800	8·3	6·39
Dec. 17	—	35	3·02	11·75	8·70	0·74	76	16	82	—	2	—	7,000	9·0	5·95
Sheep XIV—															
July 6	7	27	3·24	8·34	8·68	1·04	76	—	—	—	—	—	7,000	11·7	4·12
„ 17	6	27	3·10	8·71	8·08	0·92	71	41	55	2	1	1	4,000	8·2	4·41
Sold August, 1933.															
Sheep XV——															
July 17	16	17	2·83	6·00	5·41	0·90	47	—	—	—	—	—	6,400	8·2	6·44
Aug. 21	7	23	2·77	8·30	7·96	0·96	70	—	—	—	—	—	9,800	—	—
Oct. 19	1	30	3·09	9·70	9·30	0·96	81	26	55	2	17	0	4,800	10·7	6·43
Nov. 16	3	37	3·56	10·40	9·90	0·95	86	36	50	2	12	0	9,600	—	—
Dec. 19	2	37	3·40	10·87	9·80	0·90	86	30	53	2	15	0	8,400	8·0	6·75
Jan. 15	0	32	2·31	13·86	11·30	0·82	99	24	52	3	21	0	8,600	9·4	6·19
Feb. 12	0	32	2·85	11·23	10·40	0·93	91	24	61	2	13	0	6,400	—	—
Mar. 27	4	27	2·71	9·95	9·50	0·95	83	45	48	2	4	1	6,000	9·7	6·03
May 8	0	27	3·62	7·47	8·40	1·12	73	39	50	2	9	0	6,200	8·4	4·50
June 21	16	15	2·26	6·65	5·20	0·79	45	20	72	1	7	0	9,400	—	—
Post-mortem, 27.7.34															

TABLE VI.

COMPLETE BLOOD PICTURES. EWES. FARM B. GROUP I. PINING.

	Egg Index 100s. per gm.	*Vol. % Red Cells.*	*Av. Indiv. Cell Vol.*	*Red Blood Cells per c.mm. Mills.*	*Hæmoglobin gm. %*	*Colour Index.*	*Hæmoglobin Sh. Sc.*	*Neutrophiles. %*	*Lymphocytes. %*	*Large Monocytes. %*	*Eosinophiles. %*	*Basophiles. %*	*Total White Blood Cells per c.mm.*	*Ser. Ca.*	*Blood Inorg. Phos.*
Sheep XVI—															
July 12	25	16	3·07	5·22	5·60	1·07	49	32	63	2	3	—	6,600	8·2	3·77
„ 26	15	12	2·51	4·78	4·91	1·03	43	40	56	1	3	—	6,400	—	—
Aug. 23	13	18	2·93	6·14	6·22	1·01	54	19	76	2	3	—	5,000	7·8	4·50
Oct. 11	10	31	3·81	8·14	8·08	0·99	71	32	60	4	4	—	7,200	9·1	6·75
Nov. 9	6	35	3·80	9·22	9·00	0·98	79	31	61	3	5	—	8,600	9·4	7·42
Dec. 18	6	30	2·67	11·25	11·70	1·04	102	39	53	2	6	—	9,600	—	—
Jan. 24	12	28	3·37	8·31	8·40	1·01	73	33	60	1	6	—	11,200	9·2	7·67
Feb. 26	27	23	3·69	6·23	6·86	1·10	60	33	57	2	8	—	9,800	7·6	4·85
Sheep XVII—															
July 12	2	13	3·30	3·94	4·10	1·04	36	41	50	5	4	—	10,400	9·5	—
„ 26	3	21	3·27	6·42	6·02	0·94	53	40	55	1	4	—	12,000	—	—
Aug. 23	3	23	3·89	5·92	6·20	1·05	54	24	60	2	14	—	12,000	8·0	5·77
Oct. 11	—	22	3·22	6·84	6·40	0·94	56	42	51	2	5	—	6,400	10·4	5·53
Nov. 9	2	25	2·99	8·37	8·60	1·03	75	23	64	2	11	—	9,000	10·1	5·07
Dec. 18	1	28	3·57	7·84	8·21	1·04	72	34	56	2	8	—	5,200	—	—
Jan. 24	8	25	3·67	6·81	7·05	1·04	62	41	53	1	5	—	9,400	10·6	5·63
Feb. 26	21	25	3·28	7·62	7·05	0·93	62	45	50	2	3	—	8,000	8·7	3·40

TABLE VI—(*Contd.*).

	Egg Index 100s. per gm.	*Vol. % Red Cells.*	*Av. Indiv. Cell Vol.*	*Red Blood Cells per c.mm. Mills.*	*Hæmoglobin gm. %*	*Colour Index.*	*Hæmoglobin Sh. Sc.*	*Neutrophiles. %*	*Lymphocytes. %*	*Large Monocytes. %*	*Eosinophiles. %*	*Basophiles. %*	*Total White Blood Cells per c.mm.*	*Ser. Ca.*	*Blood Inorg. Phos.*
Sheep XVIII—															
July 12	4	16	3·36	4·77	4·92	1·03	43	41	50	4	5	Odd B.	6,600	9·1	7·76
„ 26	5	16	3·22	4·97	4·65	0·94	41	35	58	2	5	—	8,400	—	—
Aug. 23	10	14	2·93	4·78	4·60	0·95	40	39	57	2	2	—	4,000	7·8	5·04
Killed 24.8.33															
Sheep XIX—															
July 12	0	25	3·05	8·19	7·83	0·96	68	59	35	5	·5	·5	12,000	8·1	4·04
„ 26	0	27	3·37	8·02	8·21	1·02	72	26	65	4	5	—	11,400	—	—
Aug. 23	0	28	3·26	8·58	8·31	0·97	73	52	42	3	3	—	12,400	8·0	4·33
Oct. 11	3	27	3·34	8·09	7·68	0·95	67	30	52	2	16	—	9,200	8·3	9·50
Nov. 9	6	32	4·00	8·00	7·60	0·95	66	58	33	2	7	—	12,200	9·0	7·85
Dec. 11	5	33	2·68	12·33	10·10	0·82	88	43	51	2	4	—	10,000	—	—
Jan. 24	3	27	2·56	10·53	10·40	0·99	91	47	50	2	1	—	10,800	8·6	6·49
Died 16.2.34															
Sheep XX—															
July 12	10	24	2·71	8·86	8·08	0·91	71	41	50	1	6	2	6,600	8·7	3·48
„ 26	14	27	2·91	9·27	8·78	0·95	78	9	84	3	4	0	9,600	—	—
Aug. 23	17	28	3·04	9·22	8·88	0·96	78	20	73	2	4	1	13,800	8·4	6·43
GROUP II. NON-PINING.															
Sheep XXI—															
July 12	0	30	2·55	11·79	12·40	1·05	108	29	63	4	4	0	8,200	10·3	6·03
„ 26	1	25	2·38	10·50	10·10	0·96	88	28	61	2	7	0	5,600	—	—
Aug. 23	1	27	3·05	8·87	8·78	0·99	77	41	53	2	4	0	8,800	8·5	6·25
Oct. 11	—	22	2·50	8·80	9·10	1·03	79	49	47	2	2	0	13,200	9·8	4·44
Nov. 9	0	30	2·77	10·84	10·60	0·98	93	41	50	2	6	1	10,000	10·2	5·58
Dec. 18	12	25	2·91	8·58	7·53	0·88	66	34	60	3	3	0	5,600	—	—
Jan. 24	17	22	3·30	6·67	6·14	0·92	54	36	60	1	3	0	14,600	8·8	8·73
Feb. 26	51	20	3·84	5·21	5·50	1·06	48	54	43	1	2	0	11,600	8·6	5·00
Died 6.3.34															

TABLE VI—(*Contd.*).

	Egg Index 100s. per gm.	*Vol. % Red Cells.*	*Av. Indiv. Cell Vol.*	*Red Blood Cells per c.mm. Mills.*	*Hæmoglobin gm. %*	*Colour Index.*	*Hæmoglobin Sh. Sc.*	*Neutrophiles. %*	*Lymphocytes. %*	*Large Monocytes. %*	*Eosinophiles. %*	*Basophiles. %*	*Total White Blood Cells per c.mm.*	*Ser. Ca.*	*Blood Inorg. Phos.*
Sheep XXII—															
July 12	1	29	3·16	9·19	8·17	0·90	71	46	46	1	6	1	10,000	9·5	4·56
„ 26	0	23	2·55	9·02	8·88	0·98	78	39	47	2	12	0	14,200	—	—
Aug. 23	0	24	3·04	7·90	7·84	0·99	68	52	39	2	5	2	16,400	8·7	7·94
Sold															
Sheep XXIII—															
July 12	1	34	2·95	11·51	10·40	0·90	91	22	65	3	9	1	11,600	11·0	5·58
„ 26	0	28	2·93	9·55	9·31	0·97	81	31	57	3	9	0	7,400	—	—
Aug. 23	1	30	3·43	8·74	9·10	1·04	79	33	59	2	6	0	6,200	9·7	7·92
Oct. 11	1	27	2·49	10·85	10·80	1·00	94	30	60	2	8	0	6,000	10·9	4·00
Nov. 9	2	37	2·91	12·70	13·10	1·03	114	30	58	2	10	0	6,400	11·6	5·67
Dec. 18	1	40	3·11	12·87	13·40	1·04	117	43	51	2	4	0	9,800	—	—
Jan. 24	3	39	3·31	11·79	12·10	1·03	106	20	70	1	8	1	6,800	10·3	8·03
Feb. 26	8	35	3·82	9·17	9·70	1·06	85	38	53	2	7	0	7,600	9·1	5·40
Sheep XXIV—															
July 12	0	31	3·70	8·39	7·92	0·94	70	20	74	4	2	0	11,000	11·4	4·36
„ 26	0	27	3·16	8·54	8·39	0·98	73	9	82	2	7	0	10,400	—	—
Aug. 23	0	25	3·04	8·23	8·73	1·06	76	16	71	2	11	0	12,000	10·0	5·77

TABLE VI—(*Contd.*).

	Egg Index 100s. per gm.	*Vol. % Red Cells.*	*Av. Indiv. Cell Vol.*	*Red Blood Cells per c.mm. Mills.*	*Hæmoglobin gm. %*	*Colour Index.*	*Hæmoglobin Sh. Sc.*	*Neutrophiles. %*	*Lymphocytes. %*	*Large Monocytes. %*	*Eosinophiles. %*	*Basophiles. %*	*Total White Blood Cells per c.mm.*	*Ser. Ca.*	*Blood Inorg. Phos.*
Sheep XXV—															
July 12	1	28	2·73	10·27	9·44	0·92	83	—	—	—	—	—	12,400	9·9	4·96
„ 26	—	29	2·83	10·24	9·97	0·97	87	28	60	3	9	0	10,000	—	—
Aug. 23	0	30	2·74	10·96	10·40	0·94	91	21	70	3	5	1	8,800	9·8	6·43
Oct. 11	0	34	3·27	10·40	10·00	0·96	87	25	69	3	3	0	6,200	10·5	3·97
Nov. 9	1	34	2·74	12·40	11·80	0·95	103	29	56	2	13	0	7,200	8·8	9·64
Dec. 18	0	34	3·52	9·65	11·10	1·15	97	49	41	2	8	0	13,400	—	—
Jan. 24	1	33	3·04	10·85	10·20	0·94	89	34	52	2	12	0	11,400	10·4	7·03
Feb. 26	4	—	—	—	—	—	—	36	57	1	6	0	—	9·4	4·50
GROUP III. PINING.															
Copper sulphate treatment for two months commencing July 26th.															
Sheep XXVI—															
July 12	3	21	3·36	6·26	6·65	1·06	58	39	50	3	6	2	8,800	9·2	5·15
„ 26	3	17	2·92	5·82	6·11	1·19	53	26	69	2	3	0	10,400	—	—
Aug. 23	3	23	2·81	8·20	8·25	1·01	72	23	56	2	19	0	9,000	8·7	3·65
Oct. 11	0	35	3·40	10·30	10·00	0·97	87	33	48	2	17	0	8,200	10·1	3·97
Nov. 9	1	30	2·82	10·65	11·10	1·04	97	27	57	2	13	1	7,200	10·3	6·25
Dec. 18	1	33	2·34	14·12	11·10	0·79	97	32	54	2	6	0	9,000	—	—
Jan. 24	0	31	2·76	11·24	10·60	0·94	93	31	51	2	16	0	8,600	9·1	7·18
Feb. 26	1	27	3·28	8·24	8·60	1·04	75	34	55	1	7	3	12,000	7·9	7·03
Sheep XXVII—															
July 12	7	21	3·15	6·66	5·98	0·90	52	19	73	5	3	0	7,000	10·1	3·26
„ 26	3	20	3·44	5·81	6·12	1·05	53	20	70	1	9	0	12,000	—	—
Aug. 23	6	23	3·02	7·61	7·68	1·01	67	12	74	2	12	0	10,000	9·7	5·15
Oct. 11	4	33	3·59	9·20	8·35	0·91	73	17	73	3	7	0	10,400	10·4	5·32
Nov. 9	1	37	3·58	10·33	9·70	0·94	85	20	70	2	8	0	9,000	9·5	6·24
Dec. 18	0	40	3·01	13·29	12·70	0·96	111	18	72	2	7	1	15,400	—	—
Jan. 24	1	32	2·73	11·72	10·70	0·91	93	17	74	1	8	0	10,600	10·6	6·37
Feb. 26	2	30	3·06	9·79	8·10	0·83	71	11	80	2	7	0	10,600	9·6	5·82

TABLE VI—(*Contd.*).

Copper sulphate treatment for two months commencing July 26th.

	Egg Index 100s. per gm.	*Vol. % Red Cells.*	*Av. Indiv. Cell Vol.*	*Red Blood Cells per c.mm. Mills.*	*Hæmoglobin gm. %*	*Colour Index.*	*Hæmoglobin Sh. Sc.*	*Neutrophiles. %*	*Lymphocytes. %*	*Large Monocytes. %*	*Eosinophiles. %*	*Basophiles. %*	*Total White Blood Cells per c.mm.*	*Ser. Ca.*	*Blood Inorg. Phos.*
Sheep XXVIII—															
July 12	10	22	3·51	6·26	6·22	0·99	54	51	42	5	1	1	7,800	8·7	4·79
„ 26	13	23	3·03	7·59	7·57	1·00	66	50	45	1	3	1	6,000	—	—
Aug. 23	—	22	2·83	7·78	7·98	1·03	70	37	59	2	2	0	7,000	9·2	3·65
Sold															
Sheep XXIX—															
July 12	—	18	3·12	5·78	5·95	1·03	52	36	54	5	5	0	12,000	9·1	5·72
„ 26	3	22	3·57	6·17	6·80	1·10	59	49	44	2	5	0	7,200	8·2	5·15
Died. *Post-mortem* 2.8.33															
Sheep XXX—															
July 12	16	—	—	7·53	7·88	1·05	69	42	50	6	2	0	7,000	10·0	4·02
„ 26	5	28	3·75	7·46	7·76	1·04	68	20	69	3	7	1	7,000	—	—
Aug. 23	11	30	3·34	8·99	8·73	0·97	76	28	64	4	4	0	6,400	9·4	7·34
Oct. 11	4	29	3·24	8·95	9·20	1·03	80	38	57	3	2	0	5,000	9·8	4·19
Nov. 9	2	31	3·45	9·00	9·40	1·04	82	36	56	2	6	0	7,000	7·8	6·68
Dec. 18	6	32	2·82	11·33	13·00	1·15	114	24	69	2	5	0	5,800	—	—
Jan. 24	8	34	3·43	9·92	9·94	1·00	87	23	73	1	3	0	8,600	9·5	6·08
Feb. 26	4	35	3·49	10·03	10·10	1·01	88	35	60	1	4	0	6,200	9·3	3·90

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Appendix.

Egg Index	Hundreds per gramme fæces.
Volume per cent. of Red Cells ... (Hæmatocrite)	Normal sheep = 33.
Average Individual Cell Volume (Cu. cms. $\times 10^{-11}$)	Normal sheep = 3·07.
Red Blood Cells	Millions per cubic millimetre.
Hæmoglobin	...Grammes per 100 c.c. blood.
Sheep Scale	11·45 grammes = 100.
Colour Index	Normal = 1·11.
Serum Calcium	Milligrammes per cent.
Blood Inorganic Phosphate ...	Milligrammes per cent.

THE DISTRIBUTION OF *BR. ABORTUS* IN THE SYSTEM OF "CARRIER" COWS

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It is an established fact that *Br. abortus* becomes localised in the udders and in the supramammary lymphatic glands of a high percentage of infected cows.

It is known also that in some cows with positive serum agglutination titres repeated examination of the milk fails to demonstrate the organism or agglutinin for it in the milk whey.

There is strong circumstantial evidence that the presence of specific agglutinins for *Br. abortus* in blood serum or milk whey is an indication that the organism is or has been very recently present in the system of the animal; elimination of the organism appears to be rapidly followed by the disappearance of the agglutinins in detectable amount.

If this be true, then the presence of agglutinins in serum or whey over a considerable period signifies the presence of the organism in the system.

Schroeder and Cotton (1916) tested by guinea-pig inoculation the tissues of a large number of naturally infected cows for the presence of *Br. abortus*. The tissues examined were the blood, spleen, liver, kidney, brain, ovary, uterus, milk, synovial fluid from various joints, nerve tissue and lymphatic glands from all parts of the body. Excepting the udders and the supramammary lymphatic glands, the organism was isolated in only one instance —from a pelvic lymphatic gland.

Birch and Gilman (1931) examined the tissues of 25 cows for the presence of *Br. abortus;* 21 cows were artificially infected and four were naturally infected. The parts examined were the spleen, udder, uterus, ovaries, oviducts, vagina mucosa, the supramammary, mediastinal, mesenteric and the head lymphatic glands. The organism was isolated from the supramammary lymphatic glands of seven out of 17 cows; in four of these animals it had not been demonstrated in the milk.

It was recovered once out of eight specimens made up of ovaries and oviducts, twice out of 19 specimens of the lymphatic glands of the head, and once from a mixed sample of bronchial and mediastinal glands. It was isolated also from the spleen of one animal (No. 259), the serum of which gave a negative agglutination reaction in a dilution of 1 : 20.

Amoss (1931) recorded the isolation of brucella from a Fallopian tube, the glands of the meso-appendix, bile, gall-bladder wall, cysts of the ovary, serous surfaces of the intestines and from

the joint fluid of human beings. He pointed out that the histories of the cases suggested that the organisms had been present for long periods in these sites.

Leavell and Amoss (1931) reported the isolation of brucella from the bile of two human beings. They consider that the gall-bladder may be an important site of infection in chronic carriers.

Roots and Ridala (1932), isolated *Br. abortus* by guinea-pig inoculation from three naturally infected cows.

Cow I.—From the udder, superficial and deep inguinal glands, spleen, thyroid gland and a knee hygroma.

Cow II.—From the udder, superficial and deep inguinal lymphatic glands, and spleen.

Cow III.—From the udder and the right superficial inguinal lymphatic gland.

Krüger (1932) isolated *Br. abortus* by guinea-pig inoculation from the muscular pillars of the diaphragm of two cattle; the animals, a bull and a heifer, failed to react to the agglutination test.

In view of the limited information available on the persistence of the organism in the tissues of naturally infected cows, it was decided to investigate further this important phase of the disease.

It was originally intended to examine only animals the histories of which were known, but it soon became evident that in order to do so it would be necessary in many instances to purchase cows of considerable value and, as the funds available for the work were limited, we were obliged finally to accept any cheap infected animals that offered.

At the beginning of the work, in addition to the inoculation of guinea-pigs with the tissues under examination, cultures were made on liver agar, but as the results obtained by the two methods were fairly similar, and with guinea-pigs somewhat more accurate, the cultural work was discontinued.

Inoculation of Guinea-Pigs

The number of guinea-pigs inoculated varied in accordance with the importance attached to the different tissues, and it was modified as the work progressed and information was obtained on the most constant sites of the infection.

The lymphatic glands were cleaned of fascia and sterilised externally by flaming with alcohol. All tissues were ground with sterile sand and suspended in normal salt solution. The suspension was allowed to stand for about one hour and the supernatant fluid used for inoculation.

The whole of the smaller lymphatic glands were used and about 80 gms. of the larger glands and of the other organs.

The guinea-pigs were caged in pairs, one inoculated intra-

peritoneally and one intramuscularly,* the dose of inoculum in each case being 2 c.c. They were killed about the 44th day after inoculation and cultures made on liver agar from each spleen irrespective of its appearance, as *Br. abortus* may at times be isolated from apparently normal spleens. The cultures were incubated for ten days under 10 per cent. CO_2 at 37°C.

An agglutination test was made of the blood serum of each guinea-pig in dilutions of 1 : 25, 1 : 50, 1 : 100 and 1 : 200 and incubated for about 20 hours at 37°C.

The antigen used in the agglutination tests for both the cow and guinea-pig bloods consisted of a 72-hour-old broth culture of *Br. abortus,* the density of which was increased by the addition of a small amount of a 48-hour-old washed agar growth in order to match its opacity with Brown's No. 1 tube.

Br. abortus was isolated from 63 per cent. of guinea-pigs inoculated with known infected material.

The blood of 14 inoculated guinea-pigs failed to give an agglutination reaction, but the organism was isolated from them by spleen culture. Twelve animals gave positive (1 : 25) agglutination reactions, but cultures of the spleen remained sterile. Hagan (1922) has shown that normal guinea-pig blood will not agglutinate *Br. abortus* in a dilution even as low as 1 : 10.

Preparation of Milk Whey.

The milk whey was obtained by centrifuging 40 c.c. of fresh milk at 3,000 r.p.m. for 20 minutes. The middle milk was pipetted off, 1 c.c. of rennet added to it and the mixture incubated for about two hours at 37°C. At the end of this time a water clear whey was present over the clot. Agglutination tests were set up with whey and abortion antigen in dilutions of 1 : 10, 1 : 25, 1 : 50, 1 : 100 and 1 : 200, and incubated for 20 hours at 37°C.

The cream and the deposit of the centrifuged milk were suspended in a small quantity of normal salt solution and 2 c.c. inoculated intramuscularly into two guinea-pigs.

Isolation of *Br. abortus* from the Milk.

The milk from each quarter of the udder of 26 cows was inoculated intramuscularly into two guinea-pigs. In five cows a pooled sample of milk from the four quarters was used for inoculation.

Br. abortus was isolated from the udders of 22 (70 per cent.) of the 31 cows tested. Out of 17 cows, the milk from each quarter of which was separately inoculated, one was infected in

*The guinea-pigs used for the milk and the bile were inoculated intramuscularly. The amount of bile used for inoculation was 0·25 c.c.

one quarter only, seven were infected in two quarters, five in three quarters and four were infected in four quarters.

The titres of the milks from which the organism was isolated varied from 1 : 10 to 1 : 200 (the highest dilution tested).

Ten samples of whey gave positive reactions to the agglutination test, but cultures of the spleen of the guinea-pigs inoculated with the corresponding samples of whole milk (cream and deposit) remained sterile. The agglutination titres of the whey samples in which this occurred were 1 : 200 three times, 1 : 50 three times, 1 : 25 three times and 1 : 10 once. This finding is presumably of little significance and merely emphasises the need for using a sufficient number of guinea-pigs, as there can be little doubt that *Br. abortus* was present in at least some of these samples of milk.

Two samples of whey gave negative agglutination reactions, yet *Br. abortus* was isolated from the guinea-pigs inoculated with the whole milk.

These results would point to the absence of any definite correlation between the serological titre of the milk and the presence of the organism, but, in a general way, as has been shown by other workers, the higher the titre of the milk the greater is the probability of it containing *Br. abortus*.

The following are the details of the examinations of the individual cows.

Guernsey Cow No. 569.

Aged 6 years. Inoculated with dead abortion vaccine on the 3.2.30. Calved normally twice and aborted third calf at the 6th month on the 12.1.31.

Agglutination titre of blood on the 1.2.31 was 1 : 100.
„ „ „ 14.3.31 „ 1 : 50.
„ „ „ 31.3.31 „ 1 : 25.
The cow was killed on the 31.3.31.

Cultures made on liver agar of the gall-bladder wall, liver, spleen, ovary, kidney and urine remained sterile.

Guinea-pigs were inoculated from the following tissues : milk (8)*, bile (4), gall bladder wall (6), liver (2), spleen (2), ovary (4), kidney (2), and urine (2).

The guinea-pig bloods were all negative to the agglutination test on the 44th day and *Br. abortus* was not isolated from any animal by culture of the spleen.

Guernsey Cow No. 576.

History unknown.
Agglutination titre of blood on 29.4.31 was 1 : 75.
„ „ „ 14.5.31 „ 1 : 100.
The cow was killed on the 14.5.31.

*The figures in brackets refer to the number of guinea-pigs inoculated.

Tissue Examined.	Number of Guinea-pigs Examined.	Results.
Liver	4	—
Spleen	4	—
Bile	4	—
G.B.W.	6	—
Kidney	2	—
Urine	2	—
Ovary	4	—
Bone-marrow	4	—
Milk : L. fore quarter	2	—
R. „ „	2	—*
L. hind „	2	—
R. „ „	2	*Br. abortus*

*One guinea-pig had a blood titre of 1 : 25, but cultures of the spleen remained sterile.

— = *Br. abortus* not isolated.

G.B.W. = Gall-bladder wall.

Guernsey Cow No. 577.

History unknown. Not in-calf.

The udder was affected with mastitis, two quarters containing a considerable quantity of blood-stained exudate, and the other quarters a watery yellow-coloured fluid.

Agglutination titre of blood on the 30.4.31 was 1 : 500.

„ „ „ 6.5.31 „ 1 : 750.

The cow was killed on the 6.5.31.

Tissue Examined.	Number of Guinea-pigs Inoculated.	Results.
Liver	2	—
Bile	2	—
G.B.W.	6	—
Kidney	2	—
Urine	2	—
Ovary	4	—
Bone-marrow	2	—
Spleen	4	—
Milk : R. fore quarter	2	—
R. hind „	2	*Br. abortus*
L. fore „	2	—
L. hind „	2	*Br. abortus*

Cultures were made from the tissues on liver agar, but the *Br. abortus* was not isolated.

Friesland Cow No. 579.

History unknown. Not in-calf.

Agglutination titre of blood on 18.5.31 was 1 : 50.

„ „ „ 26.5.31 „ incomplete 1 : 25.

The cow was killed on the 27.5.31.

Tissue Examined.	Number of Guinea-pigs Inoculated.	Results.
Liver	2	—
Bile	2	*Br. abortus*
G.B.W.	4	—
Kidney	2	—
Ovary	2	—
Bone-marrow	2	—
Spleen	2	—
Milk : L. fore quarters	2	—
R. „ „	2	—
L. hind „	2	—
R. „ „	2	—

The *Br. abortus* was isolated also from the bile by direct culture on liver agar medium.

Guernsey Cow No. 580.

Aged about 6 years. History unknown.
Agglutination titre of blood on 1.6.31 was 1 : 100.
„ „ „ 9.6.31 „ 1 : 100.
The cow was killed on the 10.6.35.

Guinea-pigs inoculated with milk (8), bile (4), gall-bladder wall (8), liver (4), spleen (6), ovary (6), kidney (4), urine (2) and bone marrow (6) gave negative results. Cultures made on liver agar from the same tissues were also negative for *Br. abortus*.

Guernsey Cow No. 581.

History unknown. About 3 months in-calf.
Agglutination titre of blood on 9.6.31 was 1 : 100.
„ „ „ 26.6.31 „ 1 : 100, partial 1 : 200.
The cow was killed on the 26.6.31.

Guinea-pigs inoculated with milk (8), bile (4), gall-bladder wall (8), liver (4), spleen (6), ovary (6), kidney (4), urine (2) and bone marrow (4) gave negative results. Cultures made on liver agar from the same tissues were also negative for *Br. abortus*.

Shorthorn Cow No. 583.

Aged 10 years. This animal had calved normally four times and had never aborted.
The four quarters of the udder were affected with mastitis.
Agglutination titre of blood on 6.8.31 was 1 : 2,000.
This cow was killed on the 6.8.31.

Guinea-pigs inoculated with the pooled inflammatory exudate from the udder (6), bile (6), gall-bladder wall (6), liver (6), spleen (6), ovary (6), kidney (2), urine (2), bone marrow (6), submaxillary lymphatic glands (4), pharyngeal glands (4), mediastinal glands (4), and mesenteric glands (4) gave negative results. Cultures made on liver agar from the same tissues were also negative for *Br. abortus*.

Guernsey Cow No. 584.

Aged 6 years. Calved normally on the 30.5.28, and on the 24.4.29. Aborted 27.4.30.

Induration of one quarter of the udder.

Agglutination titre of blood on the 15.8.31 was 1 : 800.
" " " 15.9.31 " 1 : 2,000.

The cow was killed on the 15.9.31.

Tissue Examined.	*Number of Guinea-pigs Inoculated.*	*Results.*
Liver	6	—
Bile	6	—
G.B.W.	6	—
Kidney	2	—
Ovary	6	—
Bone-marrow	6	—
Spleen	6	*Br. abortus*
Supramammary glands	4	"
Pharyngeal glands	6	"
Submaxillary glands	4	"
Mesenteric glands	4	—
Knee hygroma	4	*Br. abortus*
Uterine scraping	4	—
Milk : Composite sample...	6	*Br. abortus*

— = *Br. abortus* not isolated.

The *Br. abortus* was isolated also by direct culture from the spleen and the udder tissue.

Shorthorn Cow No. 595.

Aged 4 years. This animal was clinically affected with Johne's disease. Not in-milk.

The uterus contained two fœtuses aged about 7 months.

Cultures of the stomach contents of both fœtuses were negative for *Br. abortus*.

Agglutination titre of blood on 18.9.31 was 1 : 800.
" " " 23.9.31 " 1 : 600.

The cow was killed on the 23.9.31.

Guinea-pigs inoculated with bile (6), gall-bladder wall (6), liver (6), spleen (6), ovary (6), kidney (2), bone marrow (6), pharyngeal lymphatic glands (6), submaxillary glands (4), mediastinal and bronchial glands (4), mesenteric glands (4), iliac glands (2) and supramammary glands (4) gave negative results.

Ayrshire Cow No. 618.

History unknown. This cow was not in-milk, but a small quantity of watery secretion was obtained from each quarter of the udder

Agglutination titre of blood on 16.4.32 was 1 : 2,000.
" " " 19.4.32 " 1 : 2,000.

The cow was killed on the 26.4.32.

Tissue Examined.	*Number of Guinea-pigs Inoculated.*	*Results.*
Spleen	6	—
Liver	6	—
Bile	6	—
G.B.W.	6	—
Kidney	4	—
Urine	4	—
Ovary	6	—
Bone-marrow	4	—
Supramammary glands	6	*Br. abortus*
Pharyngeal glands	6	—
Submaxillary glands	6	—
Popliteal glands	4	—
Mediastinal glands	6	—
Mesenteric glands	6	—
Milk : R. fore quarter	2	*Br. abortus*
R. hind „	2	—
L. fore „	2	*Br. abortus*
L. hind „	2	„

Ayrshire Cow No. 617.

History unknown. Not in-calf.
Agglutination titre of blood on 15.4.32 was 1 : 2,000.
„ „ „ 18.4.32 „ 1 : 1,500.
The cow was killed on the 18.4.32.

Tissue Examined.	*Number of Guinea-pigs Inoculated.*	*Results.*
Spleen	6	—
Liver	6	—
Bile	6	—
G.B.W.	6	—
Kidney	2	—
Urine	4	—
Ovary	6	—
Bone-marrow	4	—
Supramammary glands	6	*Br. abortus*
Pharyngeal glands	6	—
Submaxillary glands	6	—
Popliteal glands	4	—
Mediastinal glands	4	—
Iliac glands	8	*Br. abortus*
Milk : R. fore quarter	2	„
L. „ „	2	„
R. hind „	2	„
L. „ „	2	—

The *Br. abortus* was isolated also by direct culture from the supramammary lymphatic gland.

Jersey Cow No. 619.

Aged. History of having recently aborted.
Three of the quarters of the udder were completely indurated.
Agglutination titre of blood on the 16.5.32 was 1 : 1,280.
„ „ „ 8.6.32 „ 1 : 500.
The cow was killed on the 8.6.32.

Tissue Examined.	Number of Guinea-pigs Inoculated.	Results.
Spleen	6	—
Liver	6	—
Bile	6	—
G.B.W.	6	—
Kidney	4	—
Urine	4	—
Ovary	6	—
Bone-marrow	4	—
Supramammary glands	6	—
Pharyngeal glands	6	—
Submaxillary glands	6	—
Mediastinal glands	6	—
Mesenteric glands	6	—
Iliac glands	4	*Br. abortus*
Milk :- One good quarter only	12	—

Guernsey Cow No. 630.

Aged 6 years. Calved normally 11.3.29. Aborted a 7-months fœtus on 19.6.30.

Calved normally on the 4.10.31. Aborted a 7-months fœtus on the 28.8.32.

Agglutination titre of blood on the 28.9.32 was 1 : 2,500.

„ „ „ 6.10.32 „ 1 : 2,500.

The cow was killed on the 6.10.32.

Tissue Examined.	Number of Guinea-pigs inoculated.	Results.
Blood	6	—
Spleen	6	—
Liver	4	—
Bile	4	—
G.B.W.	4	—
Kidney	2	—
Ovary	4	—
Bone-marrow	2	—
Supramammary glands	4	*Br. abortus*
Pharyngeal glands	6	—
Submaxillary glands	4	—
Mediastinal glands	4	—
Mesenteric glands	4	—
Iliac glands	6	*Br. abortus*
Knee hygroma fluid	2	—
Uterine scraping	2	—
Milk : L. fore quarter	2	*Br. abortus*
L. hind „	2	„
R. fore „	2	„
R. hind „	2	„

The *Br. abortus* was isolated also by direct culture from the supramammary glands.

Shorthorn Cow. "Snow."

History unknown. Not in-calf.

Agglutination titre of blood on the 28.11.32 was 1 : 2,560.

„ „ „ 12.12.32 „ 1 : 2,560.

Three of the quarters of the udder were affected with mastitis. The cow was killed on the 12.12.32.

Tissue Examined.	*Number of Guinea-pigs Inoculated.*	*Results.*
Blood	6	—
Spleen	6	—
Liver...	4	—
Bile	4	—
G.B.W.	4	—
Kidney	2	—
Urine	2	—
Ovary	4	—
Bone-marrow	2	—
Supramammary glands	4	*Br. abortus*
Pharyngeal glands	6	—
Submaxillary glands	4	—
Mediastinal glands	4	—
Hepatic glands	4	—
Mesenteric glands	4	—
Iliac glands	4	—
Uterine glands	2	—
Milk : L. fore quarter	2	*Br. abortus*
L. hind ,, ...	2	,,
R. fore ,, ...	2	—
R. hind ,, ...	2	—

Shorthorn Cow. "Star."

Aged 5 years.

History unknown. Not in-calf. Dry.

Agglutination titre of blood on the 15.11.32 was 1 : 2,000.
,, ,, ,, 1.12.32 ,, 1 : 320.
,, ,, ,, 14.12.32 ,, 1 : 80.

The cow was killed on the 14.12.32.

Guinea-pigs were inoculated with the following tissues : "strippings" from the udder (8), bile (4), gall-bladder wall (4), liver (4), spleen (6), ovary (4), kidney (2), pharyngeal lymphatic glands (6), submaxillary glands (4), mediastinal glands (4), mesenteric glands (4), supramammary glands (4), iliac glands (12), hepatic glands (2), popliteal glands (2) and scraping from the uterine mucous membrane (4). All gave negative results.

Red Cow No. 637.

This cow had never aborted. Calved normally 3 times. Seven months in-calf at time of *post-mortem* examination.

Agglutination titre of blood on the 19.7.33 was 1 : 2,000.
,, ,, ,, 25.7.33 ,, 1 : 2,000.

The cow was killed on the 25.7.33.

Tissue Examined.	Number of Guinea-pigs Inoculated.	Results.
Blood	4	—
Spleen	4	—
Liver	4	—
Bile	4	—
G.B.W.	4	—
Kidney	2	—
Ovary	4	—
Bone-marrow	2	—
Supramammary glands	4	*Br. abortus*
Pharyngeal glands	6	—
Submaxillary glands	4	—
Mediastinal glands	4	—
Hepatic glands	4	—
Mesenteric glands	4	—
Iliac glands	6	—
Fœtal stomach	2	—
Milk: L. fore quarter	2	*Br. abortus*
L. hind „	2	„
R. fore „	2	„
R. hind „	2	„

Heifer No. 642.

Aged 3 years. Aborted April, 1933. Not in-calf. Almost dry, so a composite sample of milk was used for the guinea-pig inoculations.

Agglutination titre of blood on the 26.10.33 was 1 : 10,000.

„ „ „ 15.11.33 „ 1 : 10,000.

The animal was killed on the 16.11.33.

Tissue Examined.	Number of Guinea-pigs Inoculated.	Results.
Blood	6	—
Spleen	4	*Br. abortus*
Liver	4	—
Bile	4	—
G.B.W.	2	—
Kidney	2	—
Ovary	2	—
Thyroid	4	—
Bone-marrow	2	—
Supramammary glands	4	*Br. abortus*
Pharyngeal glands	6	„
Submaxillary glands	4	—
Popliteal and precrural glands	2	—
Mediastinal glands	4	*Br. abortus*
Hepatic glands	4	—
Mesenteric glands	4	—
Iliac glands	8	*Br. abortus*
Milk: Composite sample	6	„

Shorthorn Cow No. 641.

Aborted March, 1933. Two months in-calf at time of autopsy.

Agglutination titre of blood on the 26.10.33 was 1 : 2,000.

„ „ „ 7.12.33 „ 1 : 1,000.

The cow was killed on the 7.12.33.

Tissue Examined.	Number of Guinea-pigs Inoculated.	Results.
Blood	4	—
Spleen	4	—
Liver	4	—
Bile	4	—
G.B.W.	3	—
Kidney	2	—
Urine	4	—
Ovary	2	—
Thyroid	4	—
Bone-marrow	2	—
Pillars of diaphgram	4	—
Supramammary glands	4	*Br. abortus*
Pharyngeal glands	4	—*
Submaxillary glands	4	*Br. abortus*
Popliteal glands	2	—
Mediastinal glands	4	—
Hepatic glands	3	—
Mesenteric glands	4	—
Iliac glands	8	*Br. abortus*
Milk : R. fore quarter	2	,,
R. hind ,,	2	,,
L. fore ,,	2	—
L. hind ,,	2	*Br. abortus*

The milk whey from the left fore quarter had an agglutination titre of 1 : 200 (not end-titre).

*One guinea-pig had a blood titre of 1 : 100, but cultures of the spleen remained sterile.

Shorthorn Cow No. 640.

Aborted, 21.9.33. Not in-calf at time of autopsy.

Agglutination titre of blood on 26.10.33 was 1 : 1,000.
,, ,, ,, 7.12.33 ,, 1 : 500.
,, ,, ,, 12. 1.34 ,, 1 : 50.

The cow was killed on the 12.1.34.

Tissue Examined.	Number of Guinea-pigs Inoculated.	Results.
Blood	6	—
Spleen	6	—
Liver	4	*Br. abortus*
Bile	4	—*
G.B.W.	2	—
Kidney	2	—
Urine	6	—
Ovary	2	—
Thyroid	6	—
Bone-marrow	2	—
Pillars of diaphragm	6	—
Supramammary glands	4	—
Pharyngeal glands	6	—
Submaxillary glands	4	—
Popliteal and precrural glands	4	—
Hepatic glands	2	—
Mesenteric glands	4	—
Iliac glands	14	—
Milk : L. fore quarter	2	—
L. hind ,,	2	—
R. fore ,,	2	—
R. hind ,,	2	—

The milk whey from the left fore quarter had an agglutination titre of 1 : 50.

*One guinea-pig had a blood titre of 1 : 50, but cultures of the spleen remained sterile.

Heifer No. 643.

History unknown.

Agglutination titre of blood on the 1.11.33 was 1 : 200.

„ „ „ 23.11.33 „ 1 : 1,000.

The heifer was killed on the 23.11.33.

Tissue Examined.	*Number of Guinea-pigs Inoculated.*	*Results.*
Blood	4	—
Spleen	4	—
Liver...	4	—
Bile	4	—
G.B.W.	2	—
Kidney	2	—
Ovary	2	—
Thyroid	4	—
Bone-marrow	2	—
Pillars of diaphragm	4	—
Supramammary glands	4	*Br. abortus*
Pharyngeal glands	4	—
Submaxillary glands	4	—
Popliteal and precrural glands	4	—
Mediastinal glands...	4	—
Hepatic glands	2	—
Mesenteric glands	4	—
Iliac glands	10	*Br. abortus*
Milk : R. fore quarter	2	„
R. hind „	2	—
L. fore „	2	*Br. abortus*
L. hind „	2	—

The milk whey from both the right and left hind quarters of the udder had agglutination titres of 1 : 200 (not end-titre).

Shorthorn Cow No. 645.

Aged 4 years. Calved normally, December, 1932. Aborted 1.11.33.

Agglutination titre of blood on the 9.2.34 was 1 : 1,000 (not end-titre).

„ „ „ 19.3.34 „ 1 : 2,500.

The animal was killed on the 19.3.34.

Tissue Examined.	*Number of Guinea-pigs Inoculated.*	*Results.*
Blood	6	—
Spleen	4	—
Liver...	4	—
Bile	4	—
G.B.W.	4	—
Kidney	2	—
Ovary	2	—
Thyroid	6	—
Bone-marrow	2	—
Supramammary glands	4	—
Pharyngeal glands	4	*Br. abortus*
Submaxillary glands	4	—
Popliteal and precrural glands	2	—
Mediastinal glands	6	—
Hepatic glands	2	—
Mesenteric glands	6	—
Iliac glands	14	—
Milk : composite sample	6	*Br. abortus*

Shorthorn Cow No. 644.

Aged 4 years. Calved normally 26.9.32. Aborted 27.6.33.

Agglutination titre of blood on the 9.2.34 was 1 : 2,000 (not end-titre).

„ „ „ 26.3.34 „ 1 : 5,000.

The animal was killed on the 26.3.34.

Tissue Examined.	*Number of Guinea-pigs Inoculated.*	*Results.*
Blood	6	—
Spleen	4	—
Liver	6	—
Bile	4	—
G.B.W.	4	—
Kidney	2	—
Urine	6	—
Ovary	2	—
Thyroid	6	—
Bone-marrow	2	—
Pillars of diaphragm	6	—
Supramammary glands	4	*Br. abortus*
Pharyngeal glands	4	—
Submaxillary glands	4	—
Popliteal and precrural glands	2	—
Mediastinal glands	4	—
Hepatic glands	2	—
Mesenteric glands	4	—
Iliac glands	12	—
Milk : Composite sample	8	*Br. abortus*

Shorthorn Cow No. 647.

History unknown.

Agglutination titre of blood on the 20.4.34 was 1 : 1,000.

The cow was killed on the 20.4.34.

Tissue Examined.	*Number of Guinea-pigs Inoculated.*	*Results.*
Blood	6	—
Spleen	4	—
Liver	6	—
Bile	6	—
G.B.W.	4	—
Kidney	2	—
Urine	6	—
Ovary	2	—
Thyroid	6	—
Bone-marrow	2	—
Pillars of diaphragm	6	—
Supramammary glands	4	*Br. abortus*
Pharyngeal glands	4	—
Submaxillary glands	4	—
Popliteal and precrural glands	2	—
Mediastinal glands	4	—
Hepatic glands	2	—
Mesenteric glands	4	—
Iliac glands	8	—*
Milk : L. fore quarter	2	*Br. abortus*
L. hind „	2	„
R. fore „	2	„
R. hind „	2	„

*One guinea-pig had a blood titre of 1 : 50, but cultures of the spleen remained sterile.

Guernsey Cow No. 651.

Aborted 2nd calf on the 7.4.34.
Agglutination titre of blood on the 18.4.34 was 1 : 500.
The cow was killed on the 3.5.34.

Tissue Examined.	*Number of Guinea-pigs Inoculated.*	*Results.*
Blood	8	—
Spleen	4	—
Liver	6	—
Bile	6	—
G.B.W.	4	—
Kidney	2	—
Urine	6	—
Ovary	2	—
Thyroid	6	—
Bone-marrow	2	—
Pillars of diaphragm	6	—
Supramammary glands	4	*Br. abortus*
Pharyngeal glands	4	—
Submaxillary glands	4	—
Prescapular glands	2	*Br. abortus*
Popliteal and precrural glands	2	—
Mediastinal glands	4	*Br. abortus*
Hepatic glands	2	—
Mesenteric glands	4	—*
Iliac glands	12	*Br. abortus*
Milk : L. fore quarter	2	„
L. hind „	2	„
R. fore „	2	„
R. hind „	2	„

*One guinea-pig had a blood titre of 1 : 200 (not end-titre), but cultures of the spleen remained sterile.

Cow No. 623.

Aged 8 years. Not in-calf.
This cow had produced five normal calves and had never aborted.
Agglutination titre of blood on the 20.2.34 was 1 : 640 (Test made by Mr. A. D. McEwen).
„ „ „ 24.8.34 „ 1 : 250.
The cow was killed on the 24.8.34.

Tissue Examined.	*Number of Guinea-pigs Inoculated.*	*Results.*
Blood	6	—
Spleen	4	—
Liver	6	—
Bile	6	—
G.B.W.	4	—
Kidney	2	—
Ovary	2	—
Thyroid	6	—
Bone-marrow	2	—

Tissue Examined.	*Number of Guinea-pigs Inoculated.*	*Results.*
Pillars of diaphragm	6	—*
Supramammary glands	4	*Br. abortus*
Pharyngeal glands	4	„
Submaxillary glands	4	—
Prescapular glands	4	—
Popliteal and precrural glands	2	—
Mediastinal glands	4	—
Hepatic glands	4	*Br. abortus*
Mesenteric glands	4	—
Iliac glands	8	*Br. abortus*
Milk : L. fore quarter	2	—
R. „ „	2	—
L. hind „	2	*Br. abortus*
R. „ „	2	„

*One guinea-pig had a blood titre of 1 : 25, but cultures of the spleen remained sterile.

Shorthorn Cow No. 715.

Aged 4 years. Aborted a 7-months fœtus on 27.8.34.

Agglutination titre of blood on the 12.9.34 was 1 : 1,250 (not end-titre).

„ „ „ 6.10.34 „ 1 : 10,000.

The cow was killed on the 28.10.34.

Tissue Examined.	*Number of Guinea-pigs Inoculated.*	*Results.*
Blood	6	—
Spleen	4	—
Liver...	6	—
Bile	6	—
G.B.W.	4	—
Kidney	2	—
Urine	6	—
Ovary	2	—
Thyroid	6	—*
Bone-marrow	2	—
Pillars of diaphragm	6	—
Supramammary glands	4	*Br. abortus*
Pharyngeal glands	4	„
Submaxillary glands	2	—
Prescapular glands	2	—
Popliteal and precrural glands	2	*Br. abortus*
Mediastinal glands	6	—
Hepatic glands	4	—
Mesenteric glands	4	—
Iliac glands	8	*Br. abortus*
Milk : L. fore quarter	2	—
L. hind „	2	*Br. abortus*
R. fore „	2	„
R. hind „	2	„

*One guinea-pig had a blood titre of 1 : 100, but cultures of the spleen remained sterile.

Guernsey Cow No. 718.

Gave birth to a full-time dead calf on 12.4.34.

Agglutination titre of blood on the 26.10.34 was 1 : 250 (not end-titre).

" " " 6.11.34 " 1 : 640.

The cow was killed on the 6.11.34.

Tissue Examined.	*Number of Guinea-pigs Inoculated.*	*Results.*
Blood	6	—
Spleen	4	—
Liver	6	—
Bile	6	—
G.B.W.	4	—
Kidney	2	—
Urine	8	—
Ovary	2	—
Thyroid	6	—
Bone-marrow	2	—
Pillars of diaphragm	6	—
Fæces	8	—
Supramammary glands	4	—
Pharyngeal glands	4	—
Submaxillary glands	4	—
Popliteal and precrural glands	4	—
Mediastinal glands	4	—
Hepatic glands	4	—
Mesenteric glands	4	—
Iliac glands	12	*Br. abortus*
Knee hygroma fluid	4	"
Milk : L. fore quarter	2	—
L. hind "	2	*Br. abortus*
R. fore "	2	"
R. hind "	2	—

Guernsey Cow No. 719.

Calved three weeks prematurely on 19.8.34.

Agglutination titre of blood on the 24.10.34 was 1 : 250.

" " " 30.10.34 " 1 : 250.

The cow was killed on the 30.10.34.

Tissue Examined.	*Number of Guinea-pigs Inoculated.*	*Results.*
Blood	6	—
Spleen	4	*Br. abortus*
Liver	6	—
Bile	6	—
G.B.W.	4	—
Kidney	2	—
Urine	8	—
Ovary	2	—
Thyroid	6	—
Bone-marrow	2	—
Pillars of diaphragm	6	—
Fæces	8	—
Supramammary glands	4	*Br. abortus*
Pharyngeal glands	4	*Br. abortus*
Submaxillary glands	4	—
Popliteal and precrural glands	4	*Br. abortus*
Mediastinal glands	4	—
Hepatic glands	4	—
Mesenteric glands	4	—
Iliac glands	8	*Br. abortus*
Milk : L. fore quarter	2	—
L. hind "	2	*Br. abortus*
R. fore "	2	"
R. hind "	2	—

Guernsey Cow No. 10.

History unknown.
Agglutination titre of blood on the 21.4.34 was 1 : 1,000.
" " " 15.11.34 " 1 : 500.
The animal was killed on the 11.12.34.

Tissue Examined.	*Number of Guinea-pigs Inoculated.*	*Results.*
Blood	6	—
Spleen	4	—
Liver	6	—
Bile	6	—
G.B.W.	4	—
Kidney	2	—
Urine	8	—
Ovary	2	—
Thyroid	6	—
Bone-marrow	2	—
Pillars of diaphragm	6	—
Fæces	8	—
Supramammary glands	4	*Br. abortus*
Pharyngeal glands	4	"
Submaxillary glands	4	"
Prescapular glands	4	—
Popliteal and precrural glands	4	—
Mediastinal glands	4	—
Hepatic glands	4	—
Mesenteric glands	4	—
Iliac glands	8	*Br. abortus*
Milk : L. fore quarter	2	"
L. hind "	2	"
R. fore "	2	"
R. hind "	2	—

Guernsey Cow No. 725.

Aged 5 years. Aborted a 6-months fœtus on the 1.6.34.
The left fore quarter of the udder was indurated and contained no milk.
Agglutination titre of blood on the 19.11.34 was 1 : 2,000.
" " " 3.12.34 " 1 : 1,000.
The cow was killed on the 3.12.34.

Tissue Examined.	*Number of Guinea-pigs Inoculated.*	*Results.*
Blood	6	—
Spleen	4	—
Liver	4	—
Bile	6	—
G.B.W.	4	—
Kidney	2	—
Urine	8	—
Ovary	2	—
Thyroid	6	—
Bone-marrow	2	—
Pillars of diaphragm	6	—
Fæces	8	—

Tissue Examined.	Number of Guinea-pigs Inoculated.	Results.
Supramammary glands	4	*Br. abortus*
Pharyngeal glands	4	—
Submaxillary glands	4	—
Prescapular glands	4	—
Popliteal and precrural glands	4	—
Mediastinal glands	4	—*
Hepatic glands	4	—
Mesenteric glands	4	—
Iliac glands	8	*Br. abortus*
Milk : R. fore quarter	2	—
L. hind „	2	*Br. abortus*
R. „ „	2	„

*One guinea-pig had a blood titre of 1 : 50, but cultures of the spleen remained sterile.

Shorthorn Cow No. 726.

Aged 7 years. Calved normally three times.

Reacted to the agglutination test as a heifer in October, 1931.

Agglutination titre of blood on the 10.12.34 was 1 : 1,000 (not end-titre).

„ „ „ 21.1.35 „ 1 : 2,000.

The cow was killed on the 21.1.35.

Tissue Examined.	Number of Guinea-pigs Inoculated.	Results.
Blood	6	—
Spleen	4	—
Liver...	6	—
Bile	6	—
G.B.W.	4	—
Kidney	2	—
Urine	6	—
Ovary	2	—
Thyroid	6	—
Bone-marrow	2	—
Pillars of diaphragm	6	—
Fæces	8	—
Supramammary glands	4	*Br. abortus*
Pharyngeal glands	4	—
Submaxillary glands	4	—
Prescapular glands	4	—
Precrural glands	4	*Br. abortus*
Popliteal glands	4	—
Mediastinal glands	4	—
Hepatic glands	4	—
Mesenteric glands	4	—
Iliac glands	8	*Br. abortus*
Milk : Composite sample...	8	„

Shorthorn Cow No. 727.

Aged 7 years. Gave birth to dead calves on the 19.11.31 and on the 5.10.33. Reacted to the agglutination test on the 23.5.32.

Agglutination titre of blood on the 4.12.34 was 1 : 250 (not end-titre).
" " " 28.1.35 " 1 : 1,000.

The cow was killed on the 28.1.35.

Tissue Examined.	*Number of Guinea-pigs Inoculated.*	*Results.*
Blood	6	—
Spleen	4	—
Liver	6	—
Bile	6	—
G.B.W.	4	—
Kidney	2	—
Urine	6	—
Ovary	2	—
Thyroid	6	—
Bone-marrow	2	—
Pillars of diaphragm	6	—
Fæces	8	—
Supramammary glands	4	*Br. abortus*
Pharyngeal glands	4	—
Submaxillary glands	4	—
Prescapular glands	4	—
Precrural glands	4	—
Popliteal glands	4	—
Mediastinal glands	4	—
Hepatic glands	4	—
Mesenteric glands	4	—
Iliac glands	14	—
Milk : Composite sample	6	—

TABLE I.

TISSUES FROM WHICH THE *Br. abortus* HAS BEEN ISOLATED.

Tissues Examined.	*Number of Cows Examined.*	*Number of Cows Positive.*	*Percentage.*
Spleen	32	3	10·6
Liver	32	1	3
Supramammary glands	25	16	64
Pharyngeal glands	26	7	28
Submaxillary glands	26	3	12
Prescapular glands	7	1	14
Popliteal and precrural glands	19	2	10
Mediastinal glands	24	2	8
Hepatic glands	19	1	5
Iliac glands	23	13	52
Knee hygroma fluid	3	2	66
Bile	32	1	3
Left fore quarter	23	7	28
Right fore quarter	23	12	48
Left hind quarter	23	11	44
Right hand quarter*	23	15	60
Pooled milk	8	4	50

*The higher percentage of infection in the right hind quarter supports the contention of Thompson (1934) that this quarter is more often infected than the other quarters.

TABLE II.

TISSUES THAT HAVE GIVEN NEGATIVE RESULTS.

Tissue Examined.	*Number of Cows Examined.*
Gall-bladder wall	32
Kidney	32
Ovary	32
Bone-marrow	30
Mesenteric glands...	25
Urine	23
Blood	19
Thyroid glands	17
Pillars of the diaphragm...	14
Fæces	6

TABLE III.

COWS WITH KNOWN HISTORIES. SHOWING THE INTERVAL AFTER ABORTION AT WHICH THE EXAMINATIONS WERE MADE AND THE SITES FROM WHICH THE *Br. abortus* WAS ISOLATED.

Number of Cow.	*Interval between Abortion and Post-mortem Examination.*	*Sites from which Br. abortus was Isolated.*
	Months.	
725	5	Udder, supramammary and iliac lymphatic glands
719	2·3	Udder, spleen, supramammary, pharyngeal, iliac, popliteal and precrural lymphatic glands
718	7	Udder, iliac gland and knee hygroma
715	2	Udder, supramammary, pharyngeal, iliac, popliteal and precrural lymphatic glands
623*	6	Udder, supramammary, iliac, pharyngeal and hepatic lymphatic glands
644	9	Udder, and supramammary lymphatic glands
645	4·6	Udder, and pharyngeal lymphatic glands
640	3·7	Liver
641	8·3	Udder, suprammammary, iliac, and submaxillary lymphatic glands
642	6·3	Udder, spleen, supramammary, iliac, pharyngeal and mediastinal lymphatic glands
630	3·6	Udder, supramammary and iliac lymphatic glands
584	4·7	Udder, spleen, knee hygroma, supramammary, pharyngeal and submaxillary lymphatic glands
10*	7·7	Udder, supramammary, iliac, pharyngeal and submaxillary lymphatic glands
726*	38	Udder, supramammary, iliac and precrural lymphatic glands
727*	31	Supramammary lymphatic glands

*There was no history of these animals having aborted, the figure in the second column refers to the interval between the first positive agglutination test and the date of the *post-mortem* examination.

Identification of the Brucella Strains Isolated.

Each strain of brucella isolated was examined as follows :—

(1) Against a positive *Br. abortus* serum.

(2) For growth under ærobic conditions and under 10 per cent. CO_2.

(3) For the production of H_2S.

(4) For growth on Huddleson's dye media.

(1) All strains were agglutinated by a positive *Br. abortus* serum.

(2) No strain examined at the time of isolation grew under ærobic conditions. The strains obtained from cows 718 and 719 were not incubated ærobically until three-and-a-half months after isolation (fourth sub-culture), when both grew profusely; neither of these animals had been inoculated with live vaccine.

(3) *Hydrogen Sulphide Production.*

The production of hydrogen sulphide was determined by Huddleson's method using lead acetate paper. All the strains produced H_2S during the first two days, a few produced a little on the third day, and one strain produced a trace on the fourth, but none on the fifth day.

(4) *Dye Tolerance Tests.**

The dye plates were prepared according to Huddleson's method (1929) and the dyes were made up in the same dilutions as used by Wilson (1933).

The methyl violet and thionin were of German manufacture and the basic fuchsin and pyronin were obtained from an English firm.

The dilutions were as follows—

	Dilutions.		
Thionin...	1 : 60,000	...	1 : 30,000
Basic fuchsin	1 : 50,000	...	1 : 25,000
Methyl violet	1 : 100,000	...	1 : 50,000
Pyronin...	1 : 200,000	...	1 : 100,000

The plates were sown with a loop-full of a 72-hour liver broth culture and incubated for three days at 37°C. under 10 per cent. CO_2.

Thionin.

None of the strains showed any growth, in either dilution, on the thionin plates.

Basic fuchsin.

All strains grew well on the basic fuchsin plates. Approximately half of them showed less growth on the 1 : 25,000 than on

*Some of the earlier strains isolated were not tested by this method.

the 1 : 50,000 plate. The remainder of the strains showed no appreciable difference in growth between the two dilutions.

Methyl Violet.

All strains grew on the methyl violet plates, with in most instances considerably more growth on the 1 : 100,000 than on the 1 : 50,000 dilution. Four strains failed to grow on the 1 : 50,000 solution and one of these strains showed only a weak growth on the 1 : 100,00 dilution.

Pyronin.

All strains grew well on the pyronin plates, but there was little difference in the amount of growth between the two dilutions of the dye.

The results of these tests show that the strains isolated belong to the *Br. abortus* group.

Discussion.

A detailed examination has been made for the presence of *Br. abortus* in 32 naturally infected cows. The organism was isolated from one or more sites in 26 cows. Twenty-three tissues were examined, ten of which gave uniformly negative results (Table II). *Br. abortus* was isolated from 13 tissues (Table I), eight of which were lymphatic glands. In 17 cows it was isolated from sites other than, or in addition to, the udder and the supramammary lymphatic glands. In addition to the udder, therefore, the principal sites of *Br. abortus* in the carrier cow appear to be in the lymphoid tissue.

In the light of the information obtained it is clear that the range of tissues examined in some of the earlier cows was too restricted, and the number of guinea-pigs inoculated too small, to give reliable results. For example, it is probable that the organism would have been isolated from cows 583 and 595, which had blood titres of 1 : 2,000 and 1 : 600 respectively, if a more intensive examination had been made.

Two attempts only were made to isolate *Br. abortus* from the uterus; in both instances results were negative. Further examinations of this site were not made, as it appears to be generally accepted that the organism disappears from the genital tract within a relatively short period after the act of abortion or calving.

Birch and Gilman (1931) have, however, reported the isolation of *Br. abortus* from the genital mucosa or submucosa of four artificially infected cows 15, 13, 8 and 7 months, respectively, after calving. They consider that a true localisation of the organism had occurred in these animals. In view of this observation further investigation of its persistence in the genital tract of *naturally infected* cows would appear to be advisable.

The isolation of *Br. abortus* from various tissues within a short time of the occurrence of abortion might be attributed to its massive liberation into the blood stream about that time and its wide distribution in the system regarded as merely a temporary phase. This, however, can hardly be the case in the animals under discussion as the organism has been isolated from various tissues months after abortion had occurred.

It has actually been isolated from the iliac lymphatic glands in a higher percentage of cases than from three of the four quarters of the udder, and at intervals of 2, 3, 5, 6, 7 and 8 months respectively after abortion had occurred. It has also been isolated from the pharyngeal lymphatic glands in a considerable percentage of cases and at intervals varying from two to seven months after the act of abortion.

In view of these results it appears reasonable to assume that *Br. abortus* had become established in the iliac and in the pharyngeal glands of some of the cows examined.

The intimate relationship of the iliac lymphatic glands to the uterus and their high incidence of infection would appear to justify their inclusion with the udder and the supramammary lymphatic glands as predilection sites of the infection in the carrier cow.

Although, in a smaller percentage of cases, the organism was isolated from the other parts mentioned, the considerable intervals after abortion at which this was effected suggest that, in some at least of these sites, it had become permanently established.

In four cows (519, 619, 640, 727) *Br. abortus* was not demonstrated in the milk and there were no agglutinins for it in the whey, but it was isolated from other parts of the body, once from each of the following tissues: bile, liver, iliac and supramammary lymphatic glands.

The organism was isolated from knee hygromas of two animals. Its occurrence has, however, been frequently reported from one or other of the pathological conditions affecting the joints of cattle, but whether or not it is the primary cause of the lesions has yet to be determined. It would appear from the evidence available that the majority of these joint affections are traumatic in origin, *Br. abortus* being merely a casual invader carried there in the blood stream.

The isolation of the organism from the liver and the bile (cows 579 and 640 respectively) is of interest as its elimination by this route would cause infection of the pastures and byres. It is a noteworthy fact that in both of these animals the blood titre was rapidly falling, presumably as the result of the elimination of the organism from the system.

The fall in the blood titre of " Star " (page 201) was considerably more rapid than that usually observed in animals which overcome the infection, but the fact that the organism was not

isolated from any of the tissues examined, although a considerable number of guinea-pigs were used, confirms to some extent the results of the agglutination tests.

Two animals (Nos. 642 and 715) had blood titres of 1 : 10,000 and subsequent examination proved them to be heavily infected.

Mitchell and Duthie record the case of a *naturally infected* cow which had an agglutination titre of 1 : 28,000 for nearly a year.

Gilman mentions a *naturally infected* cow with the remarkably high titre of 1 : 81,920 and with a strong partial reaction in the next highest dilution—1 : 163,840.

A point of considerable practical importance is that *Br. abortus* does not appear to be excreted with the urine. Samples of urine from 23 cows were examined by guinea-pig inoculation and in no instance was the organism recovered. This finding is in agreement with the observations of other workers.

Several observers have suggested that with the acquisition of further knowledge on brucella infection it may be possible to control the disease by removing from herds those animals only that have a positive milk agglutination titre. Such a procedure would appear to be unsound, as it is an established fact that *Br. abortus* can occasionally be isolated from milk that has given a negative agglutination reaction. Furthermore, it is generally recognised that a considerable percentage of cows with negative milk titres have positive blood titres and, although they may not carry the organism in the udder, they are nevertheless potential spreaders of the infection.

The observations here recorded show that *Br. abortus* may be widely distributed in the bodies of naturally infected cows long after the act of abortion has occurred.

As the animals examined were bought at random it appears reasonable to regard the distribution of the organism in them as a fairly true picture of its distribution in carrier cows in general.

Summary.

(1) The tissues of 32 naturally infected cows have been examined for the presence of *Br. abortus*.

(2) The organism has been isolated from 13 different sites in 26 cows; eight of the sites consisted of lymphoid tissue.

(3) *Br. abortus* was isolated from the iliac glands in 52 per cent. and from the pharyngeal lymphatic glands in 28 per cent. of the cows examined.

(4) In view of the high percentage of infection in the iliac glands and their close relationship to the uterus, it appears reasonable to regard them as predilection sites of *Br. abortus* in carrier cows.

(5) The strains of brucella isolated were subjected to the usual

identification tests and proved to be typical *Br. abortus* types.

(6) The results obtained from the inoculation of guinea-pigs with milk indicate that there is little correlation between the serological titre of the milk and the presence of the organism in it.

(7) *Br. abortus* was isolated from 63 per cent. of guinea-pigs inoculated with known infected material.

It is a pleasure to record my thanks to Mr. J. M. Collins for his assistance throughout this work.

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THE CONTROL OF PARASITIC GASTRITIS AND ENTERITIS IN SHEEP BY TREATMENT WITH COPPER SULPHATE AND NICOTINE SULPHATE.

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During the winter of 1933-34 and the following spring parasitic gastritis and enteritis were responsible in some areas of England for exceptionally heavy losses among sheep, more particularly among those animals under one year of age. It is the general practice in the South-Eastern counties to graze the sheep on permanent pasture, making it impracticable to attempt to control the disease by a system of regular movements and rotation of grazing. Outbreaks of disease were caused in the great majority of cases by the smaller species of stomach and intestinal worms, against which therapeutic treatment has been generally recognised to be of limited value. Where the sheep were heavily parasitised by the worms in question encouraging results with many flocks were nevertheless obtained in the field by dosing the animals with a mixture consisting of 5 grammes of copper sulphate and 5 cubic centimetres of a solution of 40 per cent. nicotine sulphate to every 100 cubic centimetres of water (rain or distilled), the doses recommended being: for ewes 20 c.c. to 30 c.c.; year old sheep 20 c.c.; lambs 6 to 9 months old 10 c.c. to 15 c.c.; lambs 3 to 6 months old 7 c.c. to 10 c.c.; lambs 2 to 3 months old 5 c.c. to 7 c.c. A brief account of this preliminary work has already been published (McEwen, 1934), and since the publication of these results further satisfactory reports have been gathered from extensive application of the treatment in the field.

Hall (1930) in a parasitological resumé mentions the copper sulphate and nicotine solution of Curtice as among those anthelmintics which had been found effective for the control of *Hæmonchus contortus* and the sheep hook worms. In this general resumé references to literature and details of treatment are not given. Turner (1932) treated sheep with 3 oz. of a 1·5 per cent. solution of copper sulphate to which 25 minims of 40 per cent. nicotine sulphate had been added. His experimental sheep however appeared to have been but very lightly infected with parasitic worms, making it impossible to assess the real value of the treatment. Mohler (1933) in reporting on the work of the Bureau of Animal Husbandry, Washington, makes the following statement, " Critical tests on lambs indicate that a 1 per cent. solution of copper sulphate was very effective for the removal of the common sheep stomach worms and partially effective for the removal of other nematode species. The results against tape

worms (*Monezia* spp.) were unsatisfactory." No further particulars are given. Le Roux (1930) in a summary of the anthelmintics of value for the treatment of domestic animals recommends a mixture consisting of 1 oz. of copper sulphate, 1 oz. of 40 per cent. nicotine sulphate and 3 pints of water for the treatment of sheep affected with *Ostertagia* spp., *Trichostrongylus* spp., *Cooperia* spp. and *Nematodirus spathiger,* the doses ranging from 15 c.c. to 30 c.c. for lambs and 45 c.c. for animals of 9 months and over. He states that the mixture is being tested but that the results are not too promising. Mönnig (1934) recommends the same mixture as that given by Le Roux for trichostrongylus infestations, saying that it has given fairly good results.

The purpose of the present paper is to record the results of the treatment with relatively large doses of a 5 per cent. solution of copper sulphate containing 5 per cent. of 40 per cent. nicotine sulphate, carried out on lambs kept under field conditions which nevertheless permitted certain conclusions being drawn regarding the value of the treatment.

It was essential for success to obtain animals which at some time during the course of the experiment would show distinct evidence of parasitic gastritis and enteritis and furthermore that the disease should not be caused by infestation with *Hæmonchus contortus,* as it is known that this species may be adequately controlled by the administration of copper sulphate.

Sheep graziers and shepherds on the Romney Marsh recognise that lambs wintered on the Marsh will not thrive and that a high mortality is to be expected, this knowledge has contributed to the universal practice of sending the lambs born on the Marsh to other districts at the end of the summer, where they remain until returned to the Marsh the following spring. There was reason to suspect that the unsuitability of the Romney Marsh for young animals in the late autumn and winter was due to the lambs becoming heavily infested with worm parasites, and furthermore that the smaller species of stomach and intestinal worms were primarily responsible, as at the many *post-mortem* examinations carried out by me on sheep of all ages from the Romney Marsh *Hæmonchus contortus* has but seldom been found and then only in small numbers, whereas heavy infestation with the smaller stomach and intestinal worms has frequently been encountered. This being so, it was decided to obtain an experimental flock of lambs on the Romney Marsh, because the permanent sheep pastures there appeared likely to afford the requisite conditions.

In June, 1934, 80 lambs with their ewes were obtained by arrangement with the owner and placed on three small adjacent fields in the parish of Brookland, Romney Marsh. Thirty-eight of the lambs with their ewes were confined to the largest of the

three fields. These lambs were divided according to appearance into two groups as comparable as possible, and each animal was numbered. One group was selected for treatment with the copper sulphate and nicotine sulphate mixture, and the other group left as a control. The choice of the group which received treatment was decided by the spin of a coin. The rest of the lambs and ewes were divided among the two remaining smaller fields and treated in a similar manner. The total weights of the lambs in June were as follows:—

	lbs.
Field 1. 19 lambs selected for treatment	787
Field 1. 19 selected as controls	786
Field 2. 11 lambs selected for treatment	473
Field 2. 11 lambs selected as controls	454
Field 3. 10 lambs selected for treatment	384
Field 3. 10 lambs selected as controls	394

The average weight of the treated lambs was 41·1 lbs. and of the control lambs 39·95 lbs.

From June onwards the treated portion of the flock was dosed at intervals, and both treated and control lambs were also weighed at intervals. The dosing was always carried out in the forenoon without any preliminary fasting; but during the winter, when a supplementary ration of concentrates was given, the ration was not fed until one or two hours after dosing. The ewes were removed in the middle of August, but for some time before and for a few weeks afterwards a few wether sheep grazed the pastures along with the lambs. The fields grazed by the animals were separated from one another by ditches, but during the latter part of the summer these dried out in places and the animals mixed freely together and thereafter all the lambs were run as one flock and later, as required, moved as a flock from field to field. Owing to the drying up of the boundary ditches a number of the animals unfortunately wandered, and could not be definitely traced, so that by September 23rd, when the flock was weighed and treatment given, only 36 treated lambs and 38 control lambs were available for the continuance of the experiment, but these remained constantly together until its termination in April.

In January one of the smaller paddocks was ploughed, and shortly afterwards a second small paddock ceased to be available as it was then being grazed by three horses and by this time the sheep were being fed a ration of concentrates, making it impracticable to feed them in the field where the horses were; the latter paddock was not used again by the sheep until three weeks before the termination of the experiment. To compensate for the loss of grazing an immediately adjacent field was made available, the field having been grazed by sheep up to the time it was used by the experimental lambs. Excluding the paddock

which was ploughed, the flock had approximately 40 acres of pasture to graze over during the period of the experiment.

By August 17th the treated animals had received three doses of the copper sulphate and nicotine sulphate mixture at approximately monthly intervals. During this period there was no detectable clinical difference between the treated and control animals, which was not surprising as the lamb stock of the Romney Marsh normally remains well until that date. There was, however, a difference in the average weight of the lambs in the three groups, the control animals now being on average 1·8 lbs. heavier than the treated although in June their average weight was less. On September 20th a control lamb died from the effects of parasitic worm infestation and on September 23rd when the animals were weighed the controls had falled back, being 1·1 lbs. lighter than the treated. The results of the experiment up to September 23rd are summarised in Table I.

TABLE I.

12.6.34. 40 TREATED AND 40 CONTROL LAMBS.

	Treated.			*Controls.*	
Dates when Treated.	*Amount.*	*Average Weight per Lamb.*	*No. Dead or Missing.*	*Average Weight per Lamb.*	*No. Dead or Missing.*
12.6.34	5 c.c. to 7 c.c.	41·1 lbs.	—	39·5 lbs.	—
17.7.34	5 c.c. to 7 c.c.	53·9 lbs.	—	53·1 lbs.	—
17.8.34	10 c.c.	54·6 lbs.	—	56·4 lbs.	—
23·9·34	10 c.c.	58·6 lbs.	4 missing	57·5 lbs.	1 missing and 1 dead from parasitic worm infestation

From September 23rd the more critical phase of the experiment was entered upon and from that date onwards each animal was weighed individually.

No rigidly fixed periods for treatment had been decided upon at the commencement of the experiment and as time progressed the periodicity of dosing and the quantity given was regulated by the consideration of the condition of the sheep, the aim being to control disease among the treated animals.

On September 23rd the treated and control groups appeared equally heavy, but by November 18th a distinct difference in the general appearance of the animals comprising each group could be detected and by the middle of December these differences were most marked. The fleeces of the control groups were ragged, lustreless and dirty, whereas, by comparison, those of the treated were even, clean and healthy in appearance, and a considerable number of the control animals were suffering from diarrhœa.

These obvious and marked contrasts in the condition of the two groups were confirmed by the weights of the sheep. Reference to Table II will show that the treated sheep which survived to the end of the experiment had gained by December 7·9 lbs. per head over the September weights; whereas the control animals which survived had only gained 2·9 lbs. per head during the same period. The number of deaths up to this time was insufficient to permit any conclusions being drawn regarding the value of the treatment, but it was evident from the condition of the animals that treatment had had a very beneficial effect. At this point the original control group was subdivided into three sub-groups, one of which was left without any treatment to the end of the experiment; but from December 15th onwards the other two groups received treatment, the one with copper sulphate solution, the other with nicotine sulphate solution. By this subdivision of the original control group and by the treatment of some, it was hoped to obtain a comparison of the relative anthelmintic value of copper sulphate as compared with nicotine sulphate. Furthermore, it was feared that if the deterioration of the control portion of the flock were to progress quite unchecked the expenses of the experiment would become excessive, and it was hoped that if some of the controls responded to treatment the costs would be kept down. The subdivision of the original controls was made by a shepherd who divided them into three groups of 11 sheep, the groups being as nearly comparable as possible. The selection of the groups for their respective treatments was decided by chance. Before commencing to treat the lambs one of those selected to receive nicotine sulphate was found to be in such a weak condition that it was apparent that it could not live for more than a day or two at the most and it was therefore brought to the laboratory, where it was slaughtered and examined.

By the middle of January the heavily stocked winter pastures had been grazed very bare, and as grazing alone was no longer considered sufficient for the maintenance of the animals a supplementary ration was fed, the feeding commencing on January 24th. The ration consisted of equal parts by weight of crushed oats, cotton cake, linseed cake, beans, and dried grains. This was fed to the flock in the amount of 28 lbs. per day, less than a ½ lb. per lamb. The weight of the ration fed daily remained the same until within three weeks of the termination of the experiment, when it was increased. The ration however was changed on February 18th to include three parts by weight of dried grains and one part of each of the other constitutents, and during the latter half of March some mangolds were also fed.

Particulars regarding the experimental animals from September 23rd onwards are given in Tables II and III. The weights recorded in the tables refer to those animals in each group sur-

TABLE II.

PARTICULARS OF THE TREATED LAMBS AND THE ORIGINAL CONTROL LAMBS CONSIDERED AS A SINGLE GROUP, FROM SEPTEMBER TO THE TERMINATION OF THE EXPERIMENT. 36 TREATED AND 38 CONTROL LAMBS WERE AVAILABLE ON 23.9.34.

Treated.						*Controls.*
Dates when Treated. (See also Table I.)	*Amount.*	*No. of Deaths and Dates.*	*Dates when weighed.*	*Average Gain or Loss in Weight per Animal surviving to the end of the Experiment.*	*Average Gain or Loss in Weight per Animal surviving to the end of the Experiment.*	*No. of Deaths and Dates.*
20.10.34	10 c.c.		20.10.34	+ 2·2 lbs.	+ 0·2 lbs.	Two, between 20.10.34 and 18.11.34
18.11.34	17 c.c.	One between 20.10.34 and 18.11.34	18.11.34	+ 5·3 lbs.	+ 3·7 lbs.	One, 26.11.34
29.11.34	20 c.c.	One, 18.11.34				One, 1.12.34
						One, 3.12.34
15.12.34	18 c.c.	One, 29.11.34	15.12.34	+ 7·9 lbs.	+ 2·9 lbs.	One, 15.12.34
29.12.34	10 c.c.					One, 20.12.34
12.1.35	17 c.c.		15.1.35	+ 5·6 lbs.	— 0·3 lbs.	One, 26·12·34
26.1.35	15 c.c.					One, 29.12.34
10.2.35	15 c.c.		10.2.35	+ 6·7 lbs.	— 3·2 lbs.	Four, week ending 12.1.35
24.2.35	20 c.c.					One, 22.1.35
16.3.35	20 c.c.		25.3.35	+ 11·5 lbs.	— 1·6 lbs.	One, 3.2.35
						One, 17.2.35
		One, 1.4.35 but not due to parasitic worm infestation	9.2.35	+ 17·5 lbs.	+ 1·7 lbs.	One, 22.2.35
						One, 18.3.35

The average weight in September is taken as the basis from which gains or losses in weight are recorded.

viving to the end of the experiment, with the exception of one treated lamb which died in April from some cause other than parasitic worm infestation and whose weight was included with the others of its group.

In Table II the sheep treated with copper sulphate and nicotine sulphate mixture are compared with the entire original control group, the fact that certain of the latter received some other form of treatment from December 15th onwards being meantime disregarded. It will be seen that up to November 18th treatment had been carried out at approximately monthly periods but that after that date the sheep were dosed more frequently, at intervals of approximately two weeks. While still on monthly treatments two treated lambs receiving copper sulphate and nicotine sulphate died and shortly afterwards, on November 29th, a third lamb also died. Death in two instances was attributed to the effects of parasitic worm infestation. Unfortunately a *post-mortem* examination was not made on the third lamb and the cause of death cannot be stated. After November 29th no more animals in this group died from parasitic worm infestation.

When the original control animals are considered it is found that between October 20th and March 18th no less than 18 out of 38 died and in 16 cases death was attributed to parasitic worm infestation; the cause of death in the two remaining cases cannot be stated as no *post-mortem* examinations were made. It is interesting to note that the average September weight of the three lambs in the treated group which died was only 36·6 lbs., whereas the average weight of all the lambs in that group in September was 58·6 lbs. These three animals were therefore under-sized, probably the least vigorous, and possessed little resistance to parasitic worm infestation. The average September weight of the 18 original control lambs, however, was 55·2 lbs., which was only slightly less than that of the entire control group, namely 57·6 lbs. Whereas only three under-sized treated animals succumbed—a mortality of 8·3 per cent., the mortality among the original control group amounted to 47 per cent.

The treated group, except for the period between December 15th and January 12th, gained steadily in weight, whereas the control animals lost weight from November until February 3rd, and it was not until March 22nd that there was evidence that the progressive loss in weight had ceased. Though the loss shown by the treated animals in the latter part of December and the beginning of January may have been contributed to by some degree of parasitic worm infestation, yet the loss was most probably due to the pasture by this time supplying an insufficient maintenance diet, because no sooner was additional feeding commenced than the loss was arrested and the animals commenced to gain weight.

With the control sheep however the loss of weight extended

over a much longer period and was not, as in the case of the treated, at once arrested by additional feeding; indeed it was not until March that evidence of gain in weight was obtained. As the only difference in the management of the two groups consisted in the fact that one had been regularly treated for parasitic worm infestation by dosing with copper sulphate and nicotine sulphate, it may be assumed that the loss of weight shown by the other group of animals for the period November 18th to December 15th, and again from January 15th to February 10th, was attributable chiefly, if not entirely, to the effects of parasitic worm infestation and not to dietary insufficiency.

Chart I shows the average gain or loss in weight of the animals surviving to the end of the experiment in the group treated with copper sulphate and nicotine sulphate and the original control group respectively. Chart 2 shows the total gains or losses in weight from September onwards of all the animals in each of the above two groups respectively. Both illustrate in an unmistakable manner the superiority of the animals treated with nicotine sulphate and copper sulphate.

CHART I

SHOWING AVERAGE GAIN OR LOSS IN WEIGHT FOR ANIMALS SURVIVING TO THE END OF THE EXPERIMENT

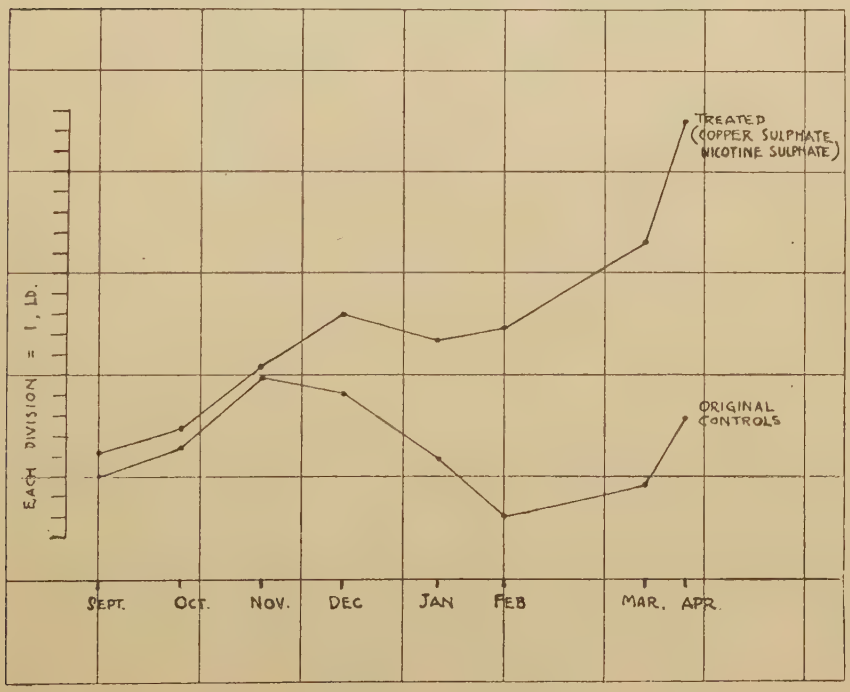
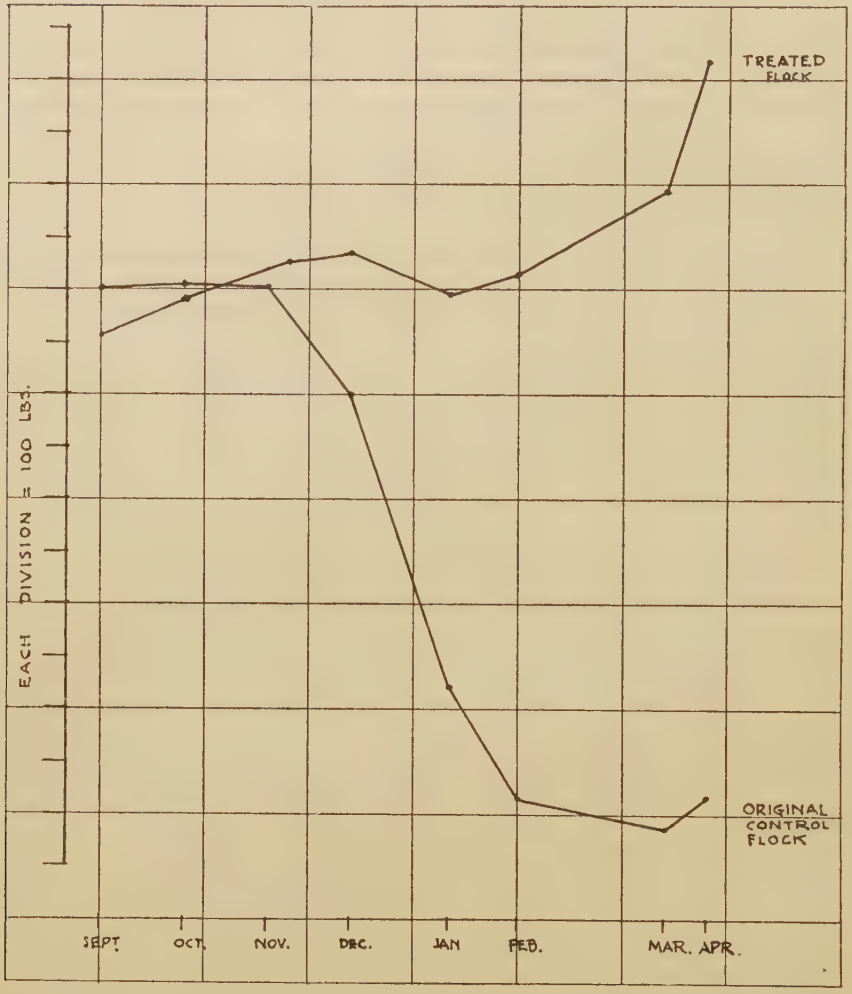


CHART II

SHOWING TOTAL GAIN OR LOSS IN WEIGHT MADE BY THE TREATED AND ORIGINAL CONTROL PORTIONS OF THE FLOCK RESPECTIVELY



On April 10th the surviving animals were sold by public auction in Ashford market, Kent. The ten best treated animals sold for £23 10s.; the ten second best treated for £22 10s.; and the 12 remaining sheep for £24 12s. As compared with these prices the ten best animals of the original control groups made £18 5s. and the ten remaining sheep £13 10s. The total value of the animals treated with copper sulphate and nicotine sulphate was £70 12s. and of the original controls £31 15s., showing that the market value of the former was more than twice that of the latter.

TABLE III.

DETAILS REGARDING THE TREATMENT OF THE ORIGINAL CONTROL ANIMALS FROM 15.12.34.

10 *Animals Treated with Nicotine Sulphate (5 per cent. solution).*				11 *Animals Treated with Copper Sulphate (5 per cent. solution).*			11 *Animals left Untreated.*	
Date when Treated.	*Amount.*	*No. of Deaths and Dates.*	*Average Gain or Loss per Animal surviving to the end of the Experiment.*	*Amount.*	*No. of Deaths and Dates.*	*Average Gain or Loss per Animal surviving to the end of the Experiment.*	*No. of Deaths and Dates.*	*Average Gain or Loss per Animal surviving the Experiment.*
15.12.34	20 c.c.			20 c.c.	One, 26.12.34		One, 20.12.34	
29.12.34	12·5 c.c.			12·5 c.c.			One, 28.12.34	
12.1.35	15 c.c.	Two, 12.1.35	— 1·6 lbs.	20 c.c.	One, 12.1.35	— 4·8 lbs.	One, 12.1.35	— 3·6 lbs.
26.1.35	15 c.c.			15 c.c.	One, 3.2.35		One, 22.1.35	
10.2.35	15 c.c.		— 3·6 lbs.	15 c.c.		— 8·4 lbs.	One, 17.2.35	— 6·8 lbs.
24.2.35	20 c.c.				One, 18.3.35		One, 22.2.35	
16.3.35	20 c.c.		+ 0·1 lbs.			— 6·1 lbs.		— 9·2 lbs.
			— 3·8 lbs.			— 2·8 lbs.		— 7 lbs.
			9.4.35			9.4.35		9.4.35

The average weight in December is taken as a basis from which gains or losses in weight were recorded.

The behaviour of the sub-groups of the original control portion of the flock is of interest and data concerning these are shown in Table III.

On December 15th the respective average weights of the sheep in the three sub-groups were 63·1 lbs. for the ten lambs subsequently treated with nicotine sulphate, 60·6 lbs. for those treated with copper sulphate, and 59·4 lbs. for those left untreated. It has already been noted that the eleventh lamb selected for treatment with nicotine sulphate was killed within a very short time of the selection being made and it has therefore been omitted from the records of the sub-groups. This lamb though weighing 52 lbs. in September was only 43 lbs. on December 15th, and had its weight been included with those of the nicotine sulphate group it would have reduced the average of the groups to 61 lbs., a figure more comparable with the average weights of the lambs in the other two groups.

Two out of ten lambs treated with nicotine sulphate died, whereas four treated with copper sulphate and six untreated animals succumbed. Furthermore, the survivors of the nicotine sulphate group did not lose weight as rapidly or to such an extent as those treated with copper sulphate or the untreated animals, and they were the only animals which in April showed an increase over their December weights. Chart III illustrates the weight curves of the three sub-groups.

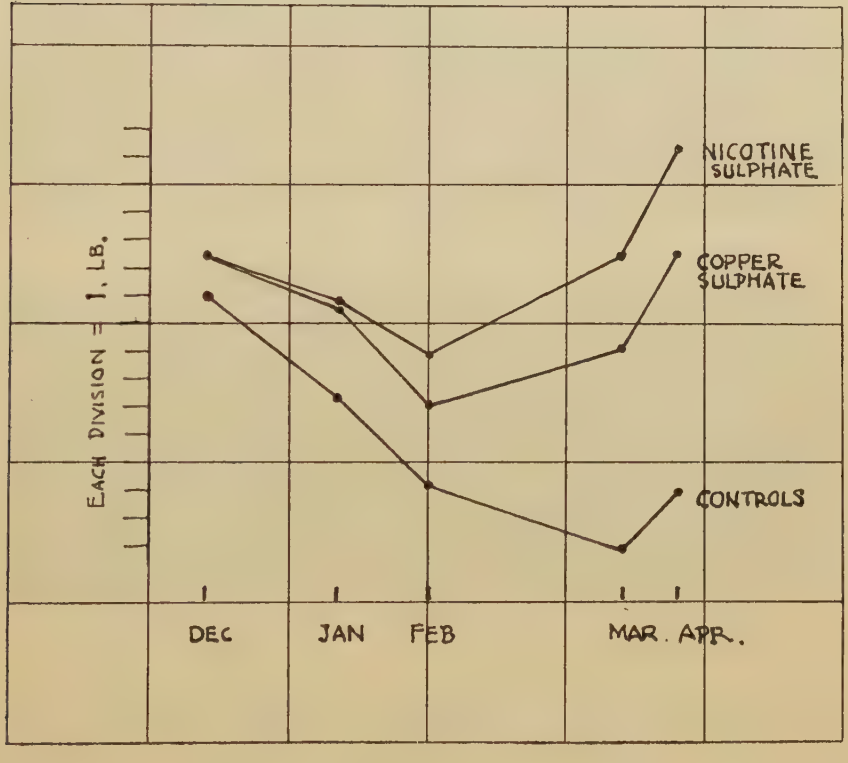
Although the numbers of experimental sheep were small in each group, the curves suggest that treatment with nicotine sulphate had a beneficial effect and was superior to copper sulphate. Moreover the beneficial effect of treatment with nicotine sulphate was detectable in the appearance of the sheep from February onwards. Treatment with copper sulphate may have been of some benefit and by March the animals so treated were healthier in appearance than the controls. The general improvement shown even by the non-treated sheep from March onwards is in accordance with what normally occurs during the spring of the year, when growth of the pastures commences, and it is most probably associated with a reduction in the extent of the worm infestation. Egg counts made at the end for March indicated that such was the case, no material difference being found in the numbers of eggs obtained from the treated or the control sheep. A comparison of the copper and nicotine sulphate mixture with nicotine sulphate alone has not been made but, should the former be the more effective, it may be due to its being more regularly carried direct to the abomasum, thus facilitating the anthelmintic action of the nicotine sulphate contained in the mixture. Ross (1933) has shown that the administration of copper sulphate solution to sheep appears to stimulate the functioning of the œsophageal groove so that in the great majority of cases the solution passes direct to the abomasum. He further suggests

that the reaction to copper sulphate might be taken advantage of to convey other anthelmintics direct to the abomasum.

Post-mortem examinations and worm counts were made whenever possible and the results of these are summarised in the appendix. A total count of the worms in the stomach and small intestine was made in the case of 16 out of the total 18 original control sheep which died after September 23rd. In

CHART III

SHOWING THE AVERAGE GAIN OR LOSS IN WEIGHT OF THE ANIMALS IN THE THREE SUB-GROUPS SURVIVING TO THE END OF THE EXPERIMENT



eleven of these animals the count was over 20,000 worms per sheep and in two of these it was above 30,000. In five instances it was 16,000 or less and in one of these cases it was as low as 2,000. A differential count was made in 13 instances and this showed that in all cases the small intestine contained a greater number of worms than the abomasum and in the former organ *Trichostrongylus vitrinus* was regularly the most numerous

of the different species found. In the abomasum *Ostertagia* spp. were found in 12 instances, *Ostertagia circumcincta* or *Ostertagia trifurcata* or both, the former being on the whole the more numerous. *Hæmonchus contortus* was recovered from one animal only, and then not until the middle of March and in the insignificant number of seven worms. It is interesting to note that ten specimens of *Hæmonchus contortus* were recovered from the treated sheep which died at the beginning of April from some cause unrelated to parasitic worm infestation. In a number of

Photographs of the experimental sheep taken at the end of March.

cases when the lungs were examined *Dictyocaulus filaria* was found in the bronchi, the numbers of these worms varying greatly in different animals and being generally associated with but limited areas of broncho-pneumonia.

From the symptoms shown by the original control animals and the *post-mortem* examinations it is evident that the sheep suffered from parasitic gastritis and enteritis, but that the enteritis was the more severe, this being indicated not only by the greater number of worms found in the small intestine but also by the large number of the control lambs which had diarrhœa and

survived. To what extent diarrhœa may be symptomatic of parasitic gastritis is uncertain, but it has not been observed in certain flocks even when the animals were emaciated and dying from heavy infestations of stomach worms, either *Hæmonchus contortus* or *Ostertagia* spp. or both, and when insignificant numbers of worms were present in the small intestine. Profuse diarrhœa is therefore regarded as a symptom indicative of parasitic enteritis rather than parasitic gastritis.

The surprisingly low worm count obtained with five of the animals, and more especially in the case of lamb No. 55, when only 2,000 were found, does not exclude parasitic worm infestation as the penultimate cause of death. All of these animals had exhibited a profuse diarrhœa for some time before death, and in the case of lamb No. 55 definite enteritis of the small intestine was noted at the *post-mortem* examination of and attributed to the effects of a recent parasitic worm infestation, a similar finding having been encountered occasionally in other animals in heavily parasitised flocks dying or killed *in extremis* after having exihibited characteristic symptoms. In such cases it is considered that the parasites, though previously present in sufficient numbers to have caused the enteritis, have been swept out of the irritated intestine.

From the evidence it is concluded that the cause of poverty, retarded growth, and death in the original control portion of the flock was parasitic worm infestation, primarily of the small intestine and to a less extent in the abomasum, and that the presence of parasitic worms in the bronchi or lungs, even when found in large numbers, was of relatively little significance, this being shown not only by the *post-mortem* findings but also by the fact that lung worms caused no serious damage in that portion of the flock where parasitic gastritis and enteritis were controlled by regular treatment of the animals with copper sulphate and nicotine sulphate. This relative unimportance of lung worm infestation by itself suggests that, where stomach and intestinal worms are controlled, specific treatment for lung worms, even were an effective treatment available, may be unnecessary.

Conclusions.

The more serious effects of nematode worm infestations of sheep and lambs grazing on permanent pasture in this country may be prevented by the repeated administration of large doses of a 5 per cent. solution of copper sulphate containing 5 per cent. of a 40 per cent. solution of nicotine sulphate.

Appendix.

Summary of examinations carried out on animals which died or were killed *in extremis*. The weights recorded were taken on the dates shown on the tables from September onwards.

Lambs Treated with Copper Sulphate and Nicotine Sulphate.

Lamb No. 34.—31 lbs., 28 lbs. This lamb died some time between October 20th and November 18th, 1934. Its death was not reported and no examination was made.

Lamb No. 46.—47 lbs., 43 lbs., 36 lbs. On November 18th, 1934 it was in a very weak condition and unable to keep up with the flock ; it received 20 c.c. of nicotine sulphate, copper sulphate mixture, showed symptoms of intoxication from nicotine poisoning shortly after treatment and died 30 minutes later. At *post-mortem* there was still a moderate amount of subcutaneous and abdominal fat ; the liver was pale, friable and fatty ; the apices of the posterior lobes of both lungs were pneumonic ; a few *Dictyocaulus filaria* were present in the bronchi ; a handful of tape worms proglottids was present in the small intestine. The worm count was :— Stomach, *Ostertagia circumcinta* 2,500, *Trichostrongylus extenuatus* 2,250, immature worms 250 ; small intestine, *Trichostrongylus vitrinus* 44,935, *Trichostrongylus colubriformis* 2,365 ; total 52,300.

Lamb No. 80.—32 lbs., 33 lbs., 32 lbs. The lamb was killed *in extremis* on November 29th, 1934. The carcase was very emaciated and anaemic ; a large number of *Dictyocaulus filaria* were present in the bronchi but the lungs presented only a few small patches of broncho-pneumonia ; the terminal portion of the ileum was thickened and the associated lymph glands appeared enlarged but no acid-fast bacilli were found in smears made from the mucous membrane of the ileum or the associated lymph glands. The worm count was :—Stomach, *Ostertagia trifurcata* 250, immature worms 250 ; small intestine, *Trichostrongylus vitrinus* 10,000, immature worms 7,000 ; total 17,500.

Lamb No. 42.—49 lbs., 51 lbs., 59 lbs., 62 lbs., 55 lbs., 58 lbs., 68 lbs. This animal was found dead on April 1st. It was in excellent condition and no lesions were found at *post-mortem* examination. Death was in no way connected with the effects of parasitic worm infestation. Analysis of the liver showed that copper was present in physiological quantities. The worm count was :—Stomach, *Ostertagia circumcincta* 200, *Ostertagia trifurcata* 50, *Trichostrongylus extenuatus* 75, immature worms 75 ; small intestine, *Trichostrongylus vitrinus* 1,490, *Trichostrongylus colubriformis* 2,000, *Haemonchus contortus* 10, immature worms 1,500 ; total 5,000.

Lambs in Original Control Group.

Lamb No. 62.—Individual weights not recorded as the animal died on September 20th, 1934, before these were taken. The stomach and intestinal contents were collected by the shepherd, mixed and forwarded to the laboratory. A differential count was not made but 11,200 small nematode worms were present together with a few tape worms. It is highly probable that only a portion of the worms was recovered.

Lamb No. 8.—73 lbs., 73 lbs.

Lamb No. 21.—50 lbs., 49 lbs. Lambs Nos. 8 and 21 died some time between October 20th and November 18th. Owing to a misunderstanding, occasioned by a change of shepherd, their deaths were not reported and no material was received for examination.

Lamb No. 24.—31 lbs., 28 lbs. The animal was found dead on the morning of November 26th and a *post-mortem* examination was not carried out until the evening of the same day. The carcase was emaciated ; the abomasum and first 15 to 20 feet of the small intestine appeared congested ; the liver was pale in colour ; the cortex of both kidneys was slightly hyperaemic. The worm count was :—Stomach, *Ostertagia circumcincta* 900, *Ostertagia trifurcata* 900, immature worms 200 ; small intestine, *Trichostrongylus vitrinus* 15,960, immature worms 10,640 ; total 28,600.

Lamb No. 64.—48 lbs., 44 lbs., 42 lbs. The animal was found dead on December 1st. The carcase was in poor condition and a few *Dictyocaulus filaria* were present in the bronchi. No specific lesions were found. The worm count was :—Stomach, *Ostertagia circumcincta* 3,000, immature worms 1,000 ; small intestine, *Trichostrongylus vitrinus* 9,300, *Trichostrongylus colubriformis* 8,370, immature worms 930 ; total 22,600.

Lamb No. 6.—68 lbs., 66 lbs., 55 lbs. The animal died on December 3rd and the stomach and intestines were received. The worm count was :—Stomach, *Ostertagia circumcincta* 3,950, *Ostertagia trifurcata* 3,150 ; small intestine, *Trichostrongylus vitrinus* 12,600, *Trichostrongylus colubriformis* 6,300, *Nematodirus filicollis* 1,050, immature worms 1,050 ; total 28,000.

Lamb No. 10.—52 lbs., 51 lbs., 50 lbs., 43 lbs. The animal was emaciated, unable to rise, and was killed on December 29th. For some time before it had been suffering from profuse diarrhœa. The carcase was very emaciated ; large numbers of *Dictyocaulus filaria* were present in the bronchi but only a small patch of one lung showed consolidation. The worm count was :—stomach, small worms 4,000, the majority being *Ostertagia circumcincta* ; small intestine 11,580, the majority being *Trichostrongylus vitrinus* ; total 16,180. Unfortunately the details of the differential count were lost.

Lamb No. 2.—69 lbs., 70 lbs., 66 lbs., 67 lbs. The animal died on December 20th, four days after it was observed to be affected with diarrhœa. The stomach and intestines only were received. The worm count was :—Stomach, *Ostertagia circumcincta* 245, immature worms 4,655 ; small intestine, *Trichostrongylus vitrinus* 4,250, immature worms 4,250 ; total 13,400.

Lamb No. 67.—56 lbs., 64 lbs., 65 lbs., 56 lbs. The animal died on December 26th ; it had been suffering from profuse diarrhœa for some time before death and had received one treatment of copper sulphate. The stomach and intestines only were received. The worm count was :—Stomach, *Ostertagia trifurcata* 2,080, immature worms 1,620 ; small intestine, *Trichostrongylus vitrinus* 2,920, *Trichostrongylus colubriformis* 350, *Nematodirus filicollis* 360, immature worms 3,650 ; total 11,000.

Lamb No. 55.—57 lbs., 59 lbs., 59 lbs., 53 lbs. On December 29th the lamb was found in a very weak condition ; it walked a few yards, fell down and could not rise, and was therefore killed. For some time before it had been suffering from profuse diarrhœa. The carcase was very emaciated though a small quantity of omental and renal fat still remained. The mucous membrane of the small intestine had a swollen, glistening and hyperæmic appearance and the organ was empty of all food material ; the contents of the cæcum and large intestine were very liquid. A few *Dictyocaulus filaria* were present in the bronchi and the apices of the anterior lobes of

both lungs showed small areas of consolidation. The worm count was :—Stomach, none ; small intestine, *Trichostrongylus vitrinus* 800, immature worms 1,200. The total worm count was 2,000.

Lamb No. 60.—61 lbs., 63 lbs., 63 lbs., 57 lbs. The animal died during the week ending January 12th. It had been treated twice with nicotine sulphate. On December 15th and 29th it had shown evidence of profuse diarrhœa. The stomach and intestines only were received and these were in a decomposed condition. The worm count was :—Stomach, small worms 9,000 ; small intestine, small worms 11,600 ; total 20,600. A differential count was not made.

Lamb No. 59.—49 lbs., 47 lbs., 47 lbs., 47 lbs. The animal was slaughtered *in extremis* on January 12th. For some time before slaughter it had been suffering from profuse diarrhœa. The carcase was very emaciated ; no appreciable quantity of abdominal fat remained. There was consolidation of the apices of the anterior lobes of both lungs and the bronchi of these parts contained masses of *Dictyocaulus filaria.* The worm count was :—Stomach, *Ostertagia circumcincta* 1,200, *Ostertagia trifurcata* 1,200 ; small intestine, *Trichostrongylus vitrinus* 12,400, *Trichostrongylus colubriformis* 9,920, immature worms 2,480 ; total 27,200.

Lamb No. 28.—54 lbs., 48 lbs., 54 lbs., 47 lbs. The animal died on January 12th. When last seen alive on December 9th it was suffering from profuse diarrhœa. It had been treated twice with nicotine sulphate. The stomachs and intestines only were received. The worm count was :—Stomach, *Ostertagia circumcincta* 3,800 ; small intestine, *Trichostrongylus vitrinus* 15,600, *Trichostrongylus colubriformis* 6,500, *Cooperia oncophora* 3,900 ; total 29,800.

Lamb No. 68.—46 lbs., 42 lbs., 54 lbs., 53 lbs. The animal died during the week ending January 12th. When seen on December 15th and again on December 29th it was suffering from profuse diarrhœa. The animal had been treated twice with copper sulphate. The stomach and intestines only were received. The worm count was :—Stomach, 8,200 small worms ; small intestine, 24,600 small worms ; total 32,800. A differential count was not made.

Lamb No. 56.—53 lbs., 56 lbs., 57 lbs., 40 lbs. The animal died on January 22nd ; when last seen on January 12th it showed evidence of suffering from diarrhœa. The stomachs and intestines only were received. The worm count was :—Stomach, *Ostertagia circumcincta* 2,100, *Trichostrongylus extenuatus* 1,050 ; small intestine, *Trichostrongylus vitrinus* 9,250, *Trichostrongylus colubriformis* 4,625, immature worms 4,625 ; total 21,650.

Lamb No. 70.—49 lbs., 51 lbs., 54 lbs., 51 lbs., 44 lbs. The animal died on February 3rd. It had been treated four times with copper sulphate and had shown evidence of suffering from diarrhœa when seen on January 12th and 26th. The stomachs and intestines only were received. The worm count was :—Stomach, *Ostertagia circumcincta* 1,500, *Ostertagia trifurcata* 2,500 ; small intestine, *Trichostrongylus vitrinus* 9,600, *Nematodirus filicollis* 2,800, immature worms 9,600 ; total 26,000.

Lamb No. 9.—47 lbs., 49 lbs., 52 lbs., 50 lbs., 40 lbs. The animal died on February 17th ; it had been suffering from diarrhœa since December. The stomachs and intestines only were received, but it was reported that large numbers of worms were present in the bronchi and that there was no pneumonia. The worm count was :—Stomach, *Ostertagia circumcincta* 2,500, *Ostertagia trifurcata* 4,000, *Trichostrongylus extenuatus* 1,500 ; small intestine, *Trichostrongylus vitrinus* 17,800, *Trichostrongylus colubriformis*

8,925, *Nematodirus filicollis* 1,785, immature worms 7,140, *Hæmonchus contortus* 7 ; total 46,507.

Lamb No. 57.—55 lbs., 60 lbs., 65 lbs., 67 lbs., 58 lbs., 43 lbs. The animal died on February 22nd. It had shown evidence of suffering from diarrhœa since December 29th. The stomachs and intestines and lungs were received at the laboratory. A moderate number of *Dictyocaulus filaria* were found in the bronchi but the lungs showed only one or two small areas of consolidation. The worm count was :—Stomach, *Ostertagia circumcincta* 300, immature worms 200 ; small intestine, *Trichostrongylus vitrinus* 3,760, *Trichostrongylus colubriformis* 3,290, immature worms 2,350 ; total 9,900.

Lamb No. 4.—60 lbs., 64 lbs., 67 lbs., 65 lbs., 65 lbs., 55 lbs., 47 lbs. The animal died on March 18th. It was noticed to be suffering from profuse diarrhœa on February 24th and it had shown evidence of slight diarrhœa since January 26th. Copper sulphate had been administered on five occasions. The carcase was very emaciated but no specific lesions were found ; however, putrefactive changes were moderately advanced when the examination was made. A small number of *Dictyocaulus filaria* were present in the bronchi. The worm count was :—Stomach, *Ostertagia circumcincta* 3,395, *Trichostrongylus extenuatus* 1,455, immature worms 4,850 ; small intestine, *Trichostrongylus vitrinus* 5,200, *Trichostrongylus colubriformis* 1,600, *Cooperia oncophora* 1,080, immature worms 2,700 ; total 20,500.

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PRELIMINARY INVESTIGATION OF A DISEASE OF SHEEP POSSESSING CERTAIN CHARACTERISTICS SIMULATING JOHNE'S DISEASE.

By G. W. Dunkin and S. E. B. Balfour-Jones.

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Our attention was first directed to the cases herein described by Mr. Donald Johnston, who described (1933) an outbreak of disease in a flock of sheep which he believed to be Johne's disease. The symptoms described were "emaciation with scouring, œdematous swelling in the submaxillary region and pallid mucous membrane." The author at first suspected worm infestation, but subsequent examination of the abomasal contents as well as those of the intestines failed to convince him that nematode parasites had any but a slight hand in the cause of the symptoms. He observed, however, that the small intestine was "intensely thickened and corrugated, and the thickening extending into the large intestine also. The mesenteric lymphatic glands were enlarged to about the size of a hazel nut, very hard to cut and ivory white on section . . . . A smear made from the mucous membrane of small intestine showed enormous numbers of acid-fast bacilli.

Recorded cases of Johne's disease in sheep are sufficiently rare to warrant further attention, and with this object in view we sought and secured the co-operation of Mr. Johnston and were able to visit the flock concerned in January, 1934. On the occasion of our visit the main flock—in charge of a shepherd of considerable experience—was grazing on some downland and a general, but not a close, examination led us to the conclusion that on the whole the sheep were in fairly good health and most of them were in excellent condition. It was learned from the shepherd that the first sign of illness that he had been able to recognise was that the affected animal separated itself from the remainder of the flock and refused to feed. Soon after this diarrhœa supervened and death followed at varying intervals of time. Some half-a-dozen of the worst cases had been collected by the owner and housed in a barn. All were obviously sick and most were in a state of emaciation, none was feeding and diarrhœa was present in all.

By way of assisting us in our attempt to carry the investigation a step further, eight sheep were purchased. Of these, five were obviously ill and would probably die and three were apparently healthy. On arrival at the laboratory they were all examined more completely than was possible during our visit to the farm. Emaciation is the only word which properly describes some of the worst cases, and the visible mucous membranes were anæmic. A curious feature seen in all the worst cases was that

the wool came out in masses following the slightest effort, in fact, by rubbing one's hand along the back the skin was left bare. The appetite varied but none would eat grain or bran, and as they were accommodated in houses with concrete floors greenstuff had to be collected for them and this they appeared to relish.

Two were selected (one apparently healthy and one sick) for the purpose of testing with johnin, and as it was proposed to make use of a variety of agents, several patches on the side of the chest wall were prepared. The following agents were injected by the intradermal route: tuberculin, avian tuberculin, synthetic johnin (1933) and johnin (1928). In all cases the dose given was 0·1 c.c. and it was repeated at the 48th hour, the final reading being taken at the 72nd hour.

In the case of the normal healthy sheep there was no reaction at any of the sites, but the diseased sheep gave a positive reaction at all patches except that which received ordinary tuberculin. This animal (S.1) was destroyed eleven days after the commencement of the test and the following *post-mortem* observations were made:—

The whole of the intestinal tract was slit up and carefully examined. Practically no food was present and it was not even necessary to wash the mucous membrane before examination. Only very slight thickening of the intestinal wall was observed and then only in places, markedly in the ileum. The ileo-cæcal valve was normal in colour and size but the cæcum showed a number of black linear markings on the mucous surface, running parallel to the long axis of the intestines. The latter part of the small intestine and the large bowel were on the whole definitely of a darker colour than is seen in normal sheep but otherwise there was little to be seen of an abnormal character. Smears from scrapings of the mucous membrane taken at various places showed on examination large numbers of acid-fast organisms.

The mesenteric lymphatic glands were for the most part slightly enlarged and on section a watery fluid escaped. This condition of the lymphatic glands was reminiscent of that seen in advanced cases of Johne's disease in cattle. Acid-fast organisms were present and easily demonstrated in the watery fluid which escaped when the glands were incised, but they were definitely less numerous than in the smears prepared from the intestinal mucous membrane.

Sections of the intestines taken at different sites were prepared and from an examination it was seen that the infection was severe. The location of the organism and the lack of histological change is reminiscent of Johne's disease in cattle. Grouping of the organisms is a feature, as it is in bovine Johne's disease, although actual clumping is not quite so marked. The picture presented, in so far as the large number of organisms present is concerned, is not unlike that seen in rat leprosy except for the grouping

tendency referred to, and also that the organisms in the disease under discussion are less slender and not so long.

Artificial Cultivation.

Attempts were made to secure cultures of the organism seen in the smear preparation and for this purpose ordinary egg slopes were used as well as the medium customarily used in these laboratories for securing growths of the organism from cases of bovine Johne's disease (1928). Before planting took place the inocula were treated with 20 per cent. antiformin, centrifuged for half-an-hour, washed in N/saline and again centrifuged. All tubes were examined every day for the first fortnight and subsequently once a month. At five months all tubes appeared to be sterile as they did at six months. In the experience of one of us (G. W. D.) primary cultures of the bacillus of Johne's disease secured from bovine lesions are obtained almost invariably in three months and on rare occasions only at four months. Assuming, therefore, that the cases now being described were those of Johne's disease, this experience was quite unprecedented. At seven months a few minute colonies were just apparent to the naked eye on three of the dozen tubes inoculated. On the ordinary egg medium no growth occurred at any time. At first the colonies seemed to increase in size while others made their appearance, and 14 days after they had first been observed subcultures were made. Smears prepared from these cultures showed that the organisms were alcohol acid fast, morphologically similar to those seen in direct smear preparations.

Subcultures grew at first with relative rapidity and were evident macroscopically in three weeks. This progress ceased, however, and growth remained stationary for almost three months, after which further subcultures were made. At the time of writing the organism appears to be growing satisfactorily, although after a magnificent effort during which the growth is apparent to the naked eye considerable reluctance is exhibited and further multiplication seems to take place less rapidly. Usually at this period it has been deemed desirable to make fresh subcultures.

Examination of Fæces.

A feature of some practical importance and of much scientific interest must be recorded concerning one of the obviously affected sheep (S.4) which was left untouched after its arrival with the others at the laboratory. Without any kind of treatment this animal apparently made a complete recovery—at any rate in bodily condition. During the time this animal had been at the laboratories it was housed away from other sheep and from all other animals. The floor of the house in which it was kept is of concrete. In view of the fact that originally it appeared to be as badly infected as S.1 it occurred to us that possibly worm infesta-

tion might have played some part in the condition originally seen. One of us (S. B.-J.) therefore examined the fæces for worm eggs during three days in March, 1934, when the average count by the sugar flotation method was 424 ova per gramme of fæces. In June this count had risen to 751 o.p.g. and in September to 1,351 o.p.g. over a period of three days. *Post-mortem* examination showed a moderately heavy infestation of the abomasum with *Hæmonchus contortus,* but little else apart from *Bunostomum trigonocephalum* in the cæcum.

Sheep 3, which died in February, 1934, however, showed a heavy infestation of the abomasum with *Hæmonchus contortus* and *Ostertagia* sp. and a large number of parasites in the small intestine, chiefly *Ostertagia* sp. probably washed through from the stomach.

Sheep 6, which died in February, 1934, also, showed a similar infestation to that found in Sheep 3 though the parasites were not so numerous.

Sheep 9, which was one of the apparently healthy group, died rather suddenly in March, 1934. A few days previously a worm egg count led one to suspect heavy worm infestation and this was proved on *post-mortem* examination. The fourth stomach and duodenum were acutely inflamed and washings of the contents of both these organs yielded a heavy crop of the same species of nematodes as were found in the previous cases.

Sheep 5, which died in July, 1934, also had had regular examination of its fæces and the egg count had shown a gradual increase from March onwards. *Post-mortem* examination showed an intensely inflamed abomasum with a heavy infestation with *Hæmonchus contortus* and a moderately heavy infestation of the duodenum with *Nematodirus* sp. and *Cooperia* sp.

It is not unlikely, therefore, that the parasitic infestation which might properly be presumed to have been heavier when the animal was first admitted, may have played some part in bringing about its emaciated condition and possibly in aggravating the disease which was found to be present in S.1.

As has already been suggested, there are certain differences in the clinical picture presented by Johne's disease in cattle and the sheep condition under discussion.

In fairly advanced cases in the sheep complete inappetence was invariably present and diarrhœa, although present, could not be described as excessive. By the use of the word " diarrhœa " it is not intended to convey the impression that the character and general appearance of the fæces was at any time comparable to the state of affairs usually present in advanced cases of bovine Johne's disease. All that one could say is that the fæces were abnormal in that there was a looseness which is not usually seen in this species and that the customary pellet form was not present.

Another feature of note is that there was an absence of

corrugation of the intestinal mucous membrane, and even thickening of the intestine was not a marked symptom.

Summary.

A disease of sheep presenting factors resembling Johne's disease of cattle has been described.

An acid-fast organism isolated from the mucous membrane of the intestine of affected sheep has been isolated and cultivated in pure culture.

Points of difference have been referred to between the condition described and Johne's disease of cattle.

Our thanks are due to Mr. Donald Johnston, M.R.C.V.S., of Lewes, who not only brought the existence of this condition to our notice but who has kindly provided us with historical information connected with the flock and has helped in other ways.

It is proposed to continue with this investigation when opportunity occurs.

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EQUINE CUTANEOUS LEISHMANIASIS: TREATMENT WITH BERBERINE SULPHATE

By S. C. J. Bennett, D.Sc., M.R.C.V.S.

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The object of this short paper is to describe what appears to be the first recorded case of equine cutaneous leishmaniasis, and treatment with Berberine Sulphate.

The patient was a native bred Sudan pony stallion aged about eight years. Smears taken from a persistent ulcer on the belly were received in the laboratory on May 11th, 1935, for examination as to the presence of cryptococci. These were not detected, but in a Giemsa-stained preparation leishmania bodies were seen.

In the original preparation the bodies showed peculiarities both as to morphology and situation. The morphological peculiarity was that the kinetoplast, so far as could be made out in so small a body, was slightly curved instead of being straight, and the situation was peculiar in that most of the intracellular bodies were contained in polynuclear leucocytes instead of in mononuclear endothelial cells.

Pending an opportunity for examining the horse itself, the original preparations were shown to Dr. E. S. Horgan, Government Bacteriologist, who confirmed that the parasites themselves, although obviously some kind of leishmania, were in this preparation not exactly similar to those found in a human oriental sore.

The horse was later sent to the laboratory for study. No history of any value accompanied it, but the sore had apparently been in existence for some considerable time, as the original smears had been sent to the laboratory on account of its persistent refusal to heal with ordinary antiseptic and astringent dressings.

The local lesion, which was about 3·5 cm. in diameter, conformed to the classical type of leishmania sore in man, having raised edges and a depressed granulating centre. Similarly its progress was quite typical. When left untreated, as was done for a period of ten days, there was the usual scab formation over the ulcerating area, the scab being easily rubbed off, revealing the underlying tissue no nearer to becoming healed.

Leishmania bodies were very plentiful in scrapings from the lesion, but with properly made scrapings from the actual granulating tissue they proved to be quite indistinguishable, either as to situation or morphology, from *L. tropica,* having a short straight kinetoplast and being, when intracellular, situated in the usual type of mononuclear cell. Dr. Horgan was kind enough to examine some of these scrapings, and admitted his inability

to distinguish them from *L. tropica* of man. Microphotographs of both intracellular and free forms are shown in figs. 1 and 2 respectively. It is thus certain that the atypical forms originally seen included in polynuclear leucocytes were undergoing degeneration and phagocytosis. The richness of these original preparations in polynuclear leucocytes would also indicate that they were not made from tissue scrapings but were merely smears from the accumulated exudate.

Owing to great pressure of other duties, it was not possible to give much attention to this case. It would, for example, have been desirable to attempt the cultivation of the parasite, if only to acquire the most probable evidence that in its flagellate form it had no distinguishing characteristics, but this could not be undertaken.

In the course of general discussion of leishmaniasis, however, Dr. Horgan mentioned recent Indian attempts at treatment with Berberine Sulphate, and it was decided to give this a trial. The technique, as described by Chopra, Dikshit and Chowhan (1932) for use in human patients, is that "One to 2 c.c. of a 1 per cent. solution of the acid sulphate is infiltrated into the margins of the sore by means of a fine hypodermic syringe. Four or more punctures are made, and care is taken to see that the infiltration is evenly spread. Injections are given once a week and the sore is dressed with ordinary surgical dressings. Frequently not more than three injections are required to bring about a complete cure, but a large number of injections may have to be given until the desired results are obtained."

Owing to the small amount of time at one's disposal, and, further, owing to the apparently easy toleration of 2 c.c. doses on the part of human patients, it was decided to give the horse double this dose; this was injected through seven or eight evenly spaced punctures around the edge of the sore. A complete cure resulted from this single treatment, but a few comments are indicated. The sore itself was so situated that "ordinary surgical dressings" could hardly be applied. It would have been possible to affix a small pad with adhesive strapping but, as daily injection of the lesion was desirable, none was applied; all that was done was to fit an "apron" of calico for the purpose of avoiding major contamination. The result obtained was therefore entirely due to the Berberine Sulphate.

The sore itself, from the day of injection, ceased to emit exudate, and consequently no further scab developed. Epithelial proliferation from the edges spread progressively over the central granulation tissue until at the end of five weeks the sore had completely healed. Consequently, however, a large persistent œdematous swelling developed. During the first week after treatment this gradually grew until it reached dimensions of about

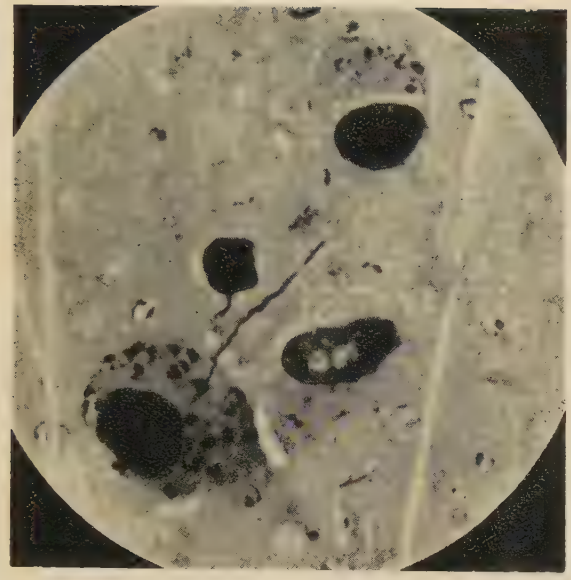

Fig. 1.—Equine Cutaneous Leishmaniasis, Intracellular Forms (× 1100).

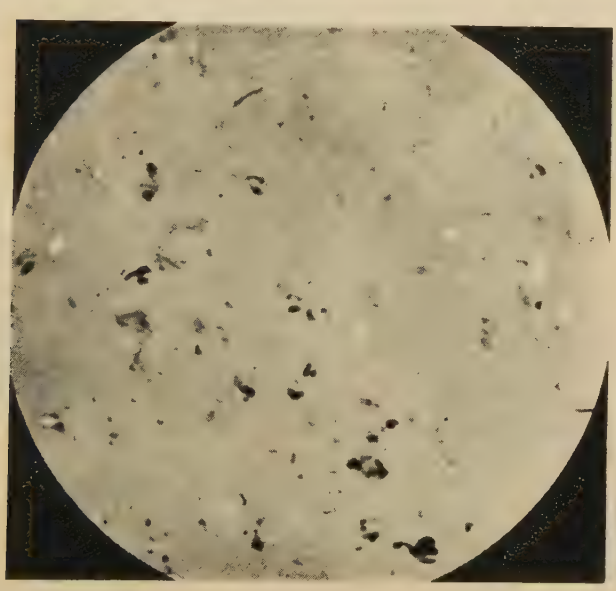

Fig. 2.—Equine Cutaneous Leishmaniasis, Free Forms.

35 by 20 cm. Since it was purely œdematous, and showed no area of necrosis or tendency to suppurate, no measures for its reduction were at first undertaken beyond exercising the patient. When, however, it showed little tendency to resolution at the end of the second week, hot fomentations were applied twice daily. The swelling was fairly obstinate in the face of this treatment, and although it ultimately disappeared it was not completely resolved until the lapse of three further weeks from the time fomentations were started. The swelling, in fact, disappeared more or less simultaneously with the sore.

Discussion.

This case is primarily of interest in being the first one of equine cutaneous leishmaniasis recorded. It is not, however, the first recorded case of equine leishmaniasis of any kind, since Richardson (1926) reported a spleen infection, tantamount to kala-azar, in a horse in Uganda.

Regarding treatment with Berberine Sulphate, it is not justifiable to draw final conclusions from the observation of a single case, but certain features of it are worthy of discussion. A complete cure followed the injection of a single set of local injections totalling 4 c.c. of a 1 per cent. solution, but a rather troublesome œdema also resulted. Judging from Chopra, Dikshit and Chowhan's findings in human oriental sore, in which multiple or even numerous sets of injections at short intervals seem to be necessary, the horse responded to treatment rather more favourably than the average human being. The very extensive and obstinate œdema that developed still further suggests that the horse may respond more readily to the action of this compound than the average human being. It has, however, to be admitted that double the normal human dose was injected and that the dependent site of the lesion may have had some bearing. It can in any case be claimed that Berberine will be worthy of further trial if any more cases of cutaneous equine leishmaniasis come to light.

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Microphotographs by Mr. N. Macdonald, Lee-Stack Memorial Laboratory, Khartoum.

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ERRATUM.

Part 3, September, 1935, p. 242, third line from the bottom, for " consequently " read " concurrently "

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